Diagnosis of human herpesviruses: Memorandum from a WHO meeting*

This memorandum reviews current methods for the diagnosis of human herpesvirus diseases, including appropriate techniques that can be utilized in the developing countries, and presents recommendations on procedures and reagents that should be developed and made available to laboratories.

Introduction

The discussions of the participants at the WHO Meeting on Diagnosis of Human Herpesvirus Infections, which was held in Berlin, Germany, in August 1990,* complemented and updated a previous WHO Meeting held in 1983 (1).

The herpesvirus family contains several important human pathogens. They possess a large number of genera, some of which have proved to be susceptible to antiviral chemotherapy. The outstanding property of herpesviruses is their ability to establish lifelong persistent infections in their hosts and to undergo periodic reactivation. It is their frequent reactivation in immunosuppressed patients which now particularly poses serious health complications. The reactivated infection may be clinically quite different from the disease caused by the primary infection. The growing epidemic of human immunodeficiency virus (HIV) infection leading to AIDS (acquired immunodeficiency syndrome) has been associated with an increased incidence of herpesvirus diseases in these immunocompromised patients.

Herpesviruses of humans include herpes simplex virus types 1 and 2 (HSV1 and HSV2), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpesvirus 6 (HHV-6). Monkey B virus (cercopithecine herpesvirus 1) is also a human pathogen.

Classification

A useful division of Herpesviridae into subfamilies is based on the biological properties of the agents (Table 1). Alphaherpesviruses (HSV, VZV) are fast-growing cytolytic viruses that tend to establish latent infections in neurons. Betaherpesviruses (CMV) are slow-growing and cytomegaglic (involving massive enlargements of infected cells) and become latent in salivary glands and kidneys. Gammaherpesviruses (EBV) infect lymphoid cells. Another herpesvirus, human B-lymphotropic virus (HBLV), has been recovered recently from patients with lymphoproliferative disorders and has been designated as human herpesvirus 6 (HHV-6); its genome resembles that of cytomegalovirus.

There is little antigenic relatedness among the Herpesviridae. Only herpes simplex virus types 1 and 2 share a significant number of common antigens. This is not surprising, since there is approximately 50% homology between those two viral genomes.

Diseases caused by Herpesviridae

Although infection is usually inapparent, a wide range of diseases are associated with the Herpesviridae. Disease caused by primary or reactivated infection by a given virus may involve different cell types and present different clinical pictures, as described below.

(1) HSV1 and HSV2 infect epithelial cells and establish latent infections in neurons. Type 1 virus is classically associated with oropharyngeal lesions and
causes recurrent attacks of “fever blisters”. Type 2 virus primarily infects the genital mucosa and is mainly responsible for genital herpes. Both viruses also cause neurological disease: HSV1 is one of the main causes of sporadic encephalitis in temperate climates.

(2) VZV causes chickenpox (varicella) on primary infection and establishes latent infection in neurons. Upon reactivation, the virus causes “shingles” (zoster). Adults infected for the first time with VZV often develop serious pneumonia. Encephalitis has also been reported as a complication of VZV infection.

(3) CMV replicates in the epithelial cells of the respiratory tract, salivary glands, kidneys and in lymphocytes. CMV may cause infectious mononucleosis and cytomegal inclusion disease (in newborns); it is an important cause of congenital defects and mental retardation.

(4) EBV replicates in epithelial cells of the oropharynx and parotid gland, and establishes latent infections in lymphocytes. It causes infectious mononucleosis and appears to be the cause of or closely associated with two human cancers, one a lymphoma and the other a carcinoma.

(5) Human herpesvirus 6 (HHV-6) infects lymphocytes. It is typically acquired in early infancy and causes exanthem subitum (roseola infantum). Target cells for latent infections and the consequences of reactivations are not known.

(6) Monkey B virus (cercopithecine herpesvirus 1) can infect humans. Such infections are rare, but those that occur result in severe neurological disease and are usually fatal.

(7) Human herpesviruses are frequently reactivated in immunocompromised patients (e.g., transplant recipients, cancer patients) and may cause severe disease, such as pneumonia or lymphomas.

(8) Herpesviruses have been linked with malignant diseases in humans and lower animals, EBV both with Burkitt’s lymphoma of African children and with nasopharyngeal carcinoma, Lucké virus with renal adenocarcinomas of the frog, Marek’s disease virus (MDV) with a lymphoma of chickens, and a number of primate herpesviruses with reticulum cell sarcomas and lymphomas in monkeys. MDV also induces atherosclerosis in chickens and current investigations have linked CMV with atherogenesis in humans.

**Diagnosis**

Each of the viruses presents its own problems and requires its own diagnostic procedures. The main procedures used for the different herpesviruses are summarized in Tables 2 to 5. Detailed methods are not given in this paper but can be found in standard laboratory manuals (2–4). Some general features have emerged; these are described below.

(1) Laboratory diagnosis of herpesvirus infections is becoming an increasingly important part of patient management as more effective means of treatment and prevention become available. The emphasis of these investigations is now directed towards diagnosis for intervention and screening for prevention. These considerations play an important role in deciding which techniques to deploy in diagnostic laboratories.

(2) The main effort in recent years has been to develop methods for rapid diagnosis, thus providing information that is useful for patient management and disease control. Methods using monoclonal antibodies for detection of viral antigen(s) directly in clinical specimens, by fluorescence microscopy or chromogenic systems, have proved highly successful and are within the scope of general diagnostic laboratories. The same methods have also been used for detection of virus in cell cultures within 24–48 hours after inoculation with clinical specimens. This is a considerable improvement in the utility of virus
isolation, traditionally regarded as the most definitive diagnostic method for all the herpesviruses (except EBV) but, because of the long incubation period often required, of limited clinical value. It will, however, in the future be important to have access to virus isolation at least in reference laboratories, specifically to aid the monitoring of drug resistance amongst the herpesviruses.

(3) Detection methods for viral DNA in clinical specimens are being advanced. It is expected that the PCR (polymerase chain reaction) method may ultimately be used for routine diagnostic purposes.

(4) Serology continues to be used for diagnosis, often in conjunction with other methods, but its role is diminishing. The reasons for this are the change in diagnostic approaches generally towards methods which do not rely on paired serum samples to demonstrate a significant rise in antibody level and doubts about the value of serological investigations for the herpesviruses in immunosuppressed patients. Concomitant antibody responses to more than one herpesvirus are also often encountered in these patients, making the interpretation of results difficult without further supportive findings. However, serology is still the mainstay of diagnosis of EBV infections. IgM antibody tests can be useful as confirmatory tests and for the diagnosis of congenital infections but are generally less useful in herpesvirus than in some other acute virus infections. Serological testing continues to be important for screening for past infection.

(5) Diagnostic reagents are now marketed commercially. The performance of commercial test kits and reagents can vary enormously and it is, therefore, advisable that laboratories using such products for virus diagnosis or antibody screening should ensure that the test performance, in terms of sensitivity and specificity, is adequate.

**Diagnosis of herpes simplex virus infections**

The laboratory diagnosis of infections caused by herpes simplex viruses (HSV-1 and HSV-2) can be made by isolation of the virus, demonstration of the presence of viral antigen or virus-specific nucleic acid sequences in appropriate specimens from patients with serious clinical forms of the infection, or serologically by demonstrating a rise in the titre of virus-specific IgG or detection of virus-specific IgM in serum samples from the patient (Table 2).

For detection of viral antigen the immunofluorescence test (IF) and enzyme-linked immunosorbent assay (EIA or ELISA) are the simplest and most reliable methods currently available, directly demonstrating the virus in clinical specimens. Detection of virus by electron microscopy is also useful, and isolation and typing of HSV may be valuable in some instances, for example for monitoring drug resistance.

Serological testing to detect IgM antibodies or a rise in IgG antibodies can be used for confirmation of primary infection or in cases with serious complications. The established methods for diagnosing cases of encephalitis are antigen detection in brain biopsies or virus isolation. Direct diagnosis may be possible with PCR on cerebrospinal fluid, but until the method is fully evaluated the diagnosis should be confirmed by demonstration of intrathecal antibody production in paired serum and CSF samples. In neonatal infections, the demonstration of virus excretion by isolation or detection of antigen by IF or EIA is most important to make the diagnosis.

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**Table 2: Diagnosis of herpes simplex virus infections**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Specimens</th>
<th>Demonstration of virus or viral products</th>
<th>Serology*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesions of skin, genitalia, eyes</td>
<td>Vesicle fluid, swab or scrape material, sera</td>
<td>Immunofluorescence test, enzyme immunoassay, and electron microscopy*</td>
<td>Complement-fixation test, enzyme immunoassay</td>
</tr>
<tr>
<td>Herpes encephalitis</td>
<td>Brain biopsy, Paired sera</td>
<td>Polymerase chain reaction*</td>
<td>Enzyme immunoassay, complement-fixation test</td>
</tr>
<tr>
<td>Neonatal herpes</td>
<td>Vesicle fluid, swab or scrape material</td>
<td>Immunofluorescence test, enzyme immunoassay, and electron microscopy*</td>
<td></td>
</tr>
</tbody>
</table>

* Using HSV1 and HSV2 specific assays for epidemiological studies.

* Can be complemented with virus isolation.

* Not yet used for routine diagnosis.
Monoclonal antibodies are preferred for use in IF and EIA tests. New techniques are being introduced for the diagnosis of diseases caused by herpes simplex virus, such as the detection of virus-specific nucleotide sequences by DNA hybridization or by PCR. These may in the future be used for routine diagnosis.

**Diagnosis of varicella-zoster virus (VZV) infections**

A variety of techniques are available for the diagnosis of VZV infections. The main uses of these techniques are summarized in Table 3.

*Electron microscopy (EM)* is simple to perform and gives results within 1–2 hours but it requires expensive equipment and cannot distinguish between HSV and VZV. Techniques (such as IF or the immunoperoxidase test (IP)) using monoclonal antibodies to probe for VZV antigen directly in clinical specimens can be used for rapid diagnosis (2–3 hours) and are more sensitive and specific than conventional cytology or histology.

*Virus isolation* is the definitive means of diagnosis but can take 3 weeks to perform. The “shell vial technique” (see below) can be used with monoclonal antibodies to allow identification of VZV in cell cultures within 2 days after inoculation.

*Serology* is commonly used for the diagnosis of VZV infections and currently is the main method for diagnosing the disease in the central nervous system. However, serological diagnosis can be complicated by apparent cross-reactivity with HSV. Testing for VZV IgM antibodies can provide additional confirmation of recent VZV infection. The most important use of serology, however, is for the determination of antibody status of at-risk patients and health care personnel to prevent VZV infection. Sensitive EIA and fluorescent antibody to membrane antigen tests (FAMA) have been developed for this purpose.

To provide a full service a laboratory offering routine diagnosis will require a direct demonstration method for rapid diagnosis of VZV disease, and a sensitive and specific test for VZV antibody status. An antigen detection system with monoclonal antibodies is most suitable for rapid diagnosis with the optional addition of electron microscopy or virus isolation if the facilities and expertise are available. An EIA is the most suitable serological test for screening for antibody status. It may be possible to provide a screening programme based on questioning for past history of varicella plus a well-optimized complement-fixation test (CFT), but only if a back-up service is available for testing CFT-negative or equivocal sera by a sensitive EIA. A regional or national reference laboratory needs to provide support and confirmatory testing for the routine laboratories including virus isolation and antigen detection. It should provide serological tests for diagnosis, such as the CFT and preferably an IgM test, and it should provide a sensitive reference test.

Table 3: *Diagnosis of varicella-zoster virus infections*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Specimens</th>
<th>Demonstration of virus or viral products</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>Vesicle fluid, scrape or swab material</td>
<td>Immunofluorescence test or immunoperoxidase test, electron microscopy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Complement-fixation test, enzyme immunoassay&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Biopsy, lavage, aspirate</td>
<td>Immunofluorescence test or immunoperoxidase test*</td>
<td>Complement-fixation test, enzyme immunoassay&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Serum&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS disease</td>
<td>Paired serum and CSF</td>
<td>Immunofluorescence test or immunoperoxidase test</td>
<td>Complement-fixation test, enzyme immunoassay&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(Biopsy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening for antibody status</td>
<td>Serum</td>
<td>Immunofluorescence test or immunoperoxidase test</td>
<td>Enzyme immunoassay, fluorescent antibody to membrane antigen test</td>
</tr>
</tbody>
</table>

<sup>a</sup> Can be complemented with isolation (conventional or rapid identification with monoclonal antibodies).

<sup>b</sup> IgM for confirmatory testing.

<sup>c</sup> Serology is of limited use for the diagnosis of pneumonia.
for antibody screening. Blood transfusion laborato-
ries do not need to screen for VZV antibody status
since the virus is not transmitted by transfusion.
However, a specific quantitative EIA or latex test is
useful for selecting units of blood with high titre
VZV antibody for the preparation of VZV immune
globulin. Fully evaluated tests or reference tests are
not yet available, so more developmental work and
evaluation will be required to establish a VZV
service.

**Diagnosis of cytomegalovirus (CMV) infections**

CMV infection is common. The virus rarely causes
disease unless the host's immune response is im-
mature (fetus) or compromised (organ transplant or
AIDS patients). Two antiviral drugs (ganciclovir
and foscarnet sodium) are available for treatment
but are toxic and should only be used after CMV
infection has definitely been diagnosed. The
methods currently used for diagnosis are summa-
ized in Table 4.

**Rapid methods of virus detection**

**DEAFF test.** Cell cultures inoculated with clinical
specimens can be fixed after overnight incubation,
reacted with monoclonal antibodies against CMV α
or β proteins and then with an immunofluorescent
conjugate. This technique is called DEAFF (detect-
ion of early antigen fluorescent foci) or shell vial
assay (because the cells may be prepared on
coverslips in shell vials).

**Leukocyte antigen detection (LAD).** Peripheral
blood mononuclear cells can be separated by dex-
tran sedimentation, centrifuged, and stained as
above with monoclonal antibodies and immuno-
peroxidase or immunofluorescent conjugates.

Both the above techniques require monoclonal
antibodies but DEAFF requires cell culture in
addition. LAD may, therefore, be more practicable
for laboratories which are not familiar with cell
cultures whereas those already using cell cultures
may prefer DEAFF because it can process samples
other than blood (e.g., urine from neonates or
bronchial lavage fluid). CMV lung infection does
not of itself progress to disease until a T-cell
immunopathological process becomes involved.
Since AIDS patients appear not to mount this
response, they do not get CMV pneumonitis and,
therefore, treatment of CMV infections of the lung
in these patients is not required, unlike the situation
in transplant recipients.

**Serology**

CMV-IgG antibodies should be detected by EIA (or
the latex agglutination test) in donors of organs or
blood, in order to exclude those who are seropos-
itive, where possible, or to match seronega-
tive recipients with only seronegative donors.

Transplant patients who are seronegative can be
monitored separately to detect seroconversion
using the same IgG methods. However, this is not
generally recommended because the diagnosis of
active infection is made more rapidly by DEAFF or
LAD, and because many transplant patients with
serious CMV disease have delayed serological re-
sponses. Testing for IgM antibodies can be used for
the diagnosis of CMV infections retrospectively and
when intervention is not immediately indicated.
Generally, however, IgG or IgM serological testing
for CMV is not recommended in immunocomp-
mised patients for the above reasons and because they
cannot reliably detect reactivations of latent infec-
tion or reinfection (which both occur in AIDS and
transplant patients). Congenital infection can be
diagnosed using CMV-IgM but sensitive and specific
assays are not readily available.

**Diagnosis of Epstein-Barr virus (EBV)
infections**

The method of choice for the diagnosis of diseases
induced by or associated with EBV differ, depend-
ing on the disease. The diseases of main importance

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Table 4: Diagnosis of cytomegalovirus infections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Specimen</th>
<th>Demonstration of virus, or viral products*</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocompromised</td>
<td>Blood</td>
<td>DEAFF or LAD</td>
<td>IgM by enzyme immunoassay</td>
</tr>
<tr>
<td>Transplant recipient</td>
<td>Bronchial lavage</td>
<td>DEAFF</td>
<td>IgG by enzyme immunoassay (or latex)</td>
</tr>
<tr>
<td>Neonate</td>
<td>Urine</td>
<td>DEAFF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ donors</td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DEAFF (detection of early antigen fluorescent foci); LAD (leukocyte antigen detection).
to the diagnostic laboratory are infectious mononucleosis (IM), Burkitt's lymphoma, and nasopharyngeal carcinoma (NPC) in endemic areas and severe disease in the immunocompromised host. Shown in Table 5 are the minimal laboratory tests required for diagnosis of these diseases. Past EBV infection is demonstrated by the presence of EBV viral capsid antigen (VCA) IgG antibodies. Presence of IgM to VCA is a sensitive and relatively specific marker of infectious mononucleosis. In teenagers and adults, IM can be diagnosed by an immediate test for heterophile antibodies. Complementary tests with high cost-benefit in IM are examinations for VCA IgG and Epstein-Barr nuclear antigen (EBNA) antibodies. Assays for EBNA antibodies and EBV DNA in biopsies should be used for diagnosis of EBV-associated malignancies.

In the reference laboratory all mentioned tests and further assays for antibodies to the various EBV antigens (EBNA 1-2, early antigen (EA)) should be available. The established antigens for the serological investigations are derived from Burkitt's lymphoma cell lines. IF assays are the most widely used for EBV serology, but commercial EIAs are also available.

A high priority should be given to the development of cheap and specific serological assays for predicting nasopharyngeal carcinoma and for the detection of IgM to viral capsid antigen.

**Diagnosis of human herpes virus type 6 (HHV-6) infections**

Since human herpesvirus type 6 (HHV-6) was only recently discovered, very little is known about its natural history and full clinical significance. Currently, there are two means of diagnosing HHV-6 infections: virus isolation and serological testing. However, neither can be recommended for routine use and they are only for consideration in reference laboratories. DNA hybridization and PCR have been employed for research purposes but have not been evaluated as diagnostic tests.

**Serological tests.** Most of the serological investigations of HHV-6 infections to date have relied on IF for IgG and IgM antibodies using acetone-fixed infected lymphocytes. EIAs have successfully been developed which are suitable as diagnostic tests but their usefulness, particularly the IgM tests, has yet to be determined. It will be important to investigate the relationships between HHV-6, CMV and EBV, since simultaneous antibody rises are often encountered with two or more of these viruses. Parallel investigations for these viruses may become routine practice in the future. For this reason it would be valuable if at least the reference laboratories acquired IF and probably EIAs for HHV-6 antibodies as investigative tools.

**Diagnosis of herpesviruses in developing countries**

The laboratory diagnosis of herpesvirus infections in developing countries has not been given sufficient prominence or priority in the past owing to the many other health problems faced by these countries. With the increasing problems of AIDS, the use of immunosuppressive therapy and the possibility of treatment, there is now a greater awareness of herpesvirus diseases and the need to introduce appropriate diagnostic services in countries where these do not yet exist. However, in deciding which tests should be introduced both practical and economic factors have to be considered. For example, since tissue culture technology is technically difficult as well as labour intensive and expensive to provide, WHO has recommended that developing countries,

<table>
<thead>
<tr>
<th>Disease</th>
<th>Specimen</th>
<th>Demonstration of virus or viral products</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious mononucleosis</td>
<td>Serum</td>
<td>IgM viral capsid antigen (immunofluorescence test, enzyme immunoassay)</td>
<td>Heterophile antibody*</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>Serum</td>
<td>IgA viral capsid antigen (immunofluorescence test)</td>
<td></td>
</tr>
<tr>
<td>Severe disease in immunocompromised patients</td>
<td>Material from lesions</td>
<td>EBNA (ACIF)c or DNA</td>
<td></td>
</tr>
</tbody>
</table>

* Rarely present below the age of 3 years.
* For identification of persons at high risk of developing nasopharyngeal carcinoma and for monitoring treatment.
* EBNA (Epstein-Barr nuclear antigen); ACIF (anti-complement immunofluorescence test).
where possible, should use rapid techniques which do not require virus replication. Techniques such as IF and EIA are widely practised for antigen detection and the detection of IgM class antibodies should also be considered in relevant diseases. In countries where information is scanty on the impact of herpesvirus diseases, serological diagnosis may have an important role for disease surveillance of the herpesviruses in different groups of patients. Such investigations may play an important role in defining national or local needs and, thus, help to target the diagnostic services provided. In order to promote herpesvirus diagnosis in developing countries, there is a need to provide technical expertise by way of workshops with a follow-up programme to enable laboratories to acquire and practise the new skills. This priority should be given to strengthening and upgrading certain laboratories in developing countries to produce working reagents for the herpesviruses of interest to that country. These reagents could then be shared on an exchange basis. Since monoclonal antibodies will probably be the most useful reagents for rapid antigen detection, workshops on hybridoma technology should be conducted in the WHO Regions.

**Recommendations**

Laboratory investigations for diagnosis and screening purposes are now important for the prevention and treatment of herpesvirus diseases. Demand for these services is likely to increase in the future in line with improved health care provisions generally and because of a growing population at risk of severe disease caused by these viruses. In order to provide such services the following are recommended:

1. A core of diagnostic procedures should be introduced in laboratories providing routine virological or microbiological services.
2. Regional or national reference laboratories should establish techniques for confirmatory testing and more specialized investigations to support the routine laboratories.
3. International standard preparations and reference reagents, including virus strains and human sera containing IgG and IgM antibodies to the herpesviruses, should be developed and quality controlled for distribution to national reference laboratories.
4. A list of monoclonal antibodies available for collaborative diagnostic studies on the human herpesviruses should be compiled.

(5) Studies on the rapid diagnosis of encephalitides caused by herpesviruses should be promoted. Such studies should emphasize the use of PCR for viral DNA detection in the cerebrospinal fluid.

**References**


**Annex**

**Suggested quality control laboratories**

The numbers refer to laboratories (see below) for diagnosis of infections caused by:

- HSV: 1, 5, 6
- CMV: 3, 4, 6
- VZV: 2, 4
- EBV: 2, 5
- HHV-6: 2, 4

1. WHO Collaborating Centre for Herpes Virus Reference and Research, The D. I. Ivanovsky Institute of Virology, Moscow, USSR.
2. WHO Collaborating Centre for Virus Reference and Research, Department of Virology, The National Bacteriological Laboratory, Stockholm, Sweden.
4. Virology Department, St Bartholomew’s Hospital Medical College, London, England.
5. Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia.
6. WHO Collaborating Centre for Virus Reference and Research, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX, USA.