Laboratory Diagnosis of Herpes Genitalis

Labordiagnose des Herpes genitalis

J. F. Chenot, H. W. Doerr, H. F. Rabenau

Summary: Herpes genitalis is caused mainly by herpes simplex virus type 2 (HSV-2) and to a lesser extent but with increasing frequency, by herpes simplex virus type 1 (HSV-1). Today, the diagnosis of genital herpes is based on laboratory methods. Serology is useful to distinguish primary infection from latent infection and for seroepidemiological investigations. Newer type-specific antibody tests based on single recombinant or purified viral antigens have a higher sensitivity and specificity for detecting anti HSV-2 antibodies. The tests also allow the discrimination between HSV-1 or -2 specific antibodies. Since serology is not able to recognize reactivation, isolation in cell culture remains the standard. If cell culture is not available or optimal transport is not possible and rapid results are needed, direct antigen detection, or in selected cases, the highly sensitive and specific PCR should be used.

Keywords: ELISA; IFT; PCR; antigen detection; PCR.

Zusammenfassung: Herpes genitalis wird hauptsächlich durch des Herpes simplex Virus Typ 2 (HSV-2) und in einem geringeren aber zunehmenden Ausmaß durch Herpes simplex Virus Typ 1 (HSV-1) verursacht. Heutzutage basiert die Diagnose des genitalen Herpes auf labortechnischen Methoden. Die Serologie wird zur Abgrenzung der primären von der latenten Infektion und für seroepidemiologische Studien eingesetzt. Neuere typenspezifische Antikörpertests welche auf einzelnen rekombinanten oder gereinigten Virusantigenen basieren, zeigen eine höhere Sensitivität und Spezifität für den HSV-2-Antikörpernachweis und ermöglichen eine typenspezifische Antikörperbestimmung. Die Serologie ist für die Diagnose einer Reaktivierung nicht geeignet, hier bleibt die Isolierung in der Zellkultur der Standard. Falls eine Zellkultur nicht verfügbar oder ein optimaler Probentransport nicht gewährleistet und ein rasches Ergebnis erforderlich ist, wird der direkte Antigennachweis oder in ausgewählten Fällen, die hoch sensitive und spezifische PCR eingesetzt.

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Schlüsselwörter: ELISA; IFT; PCR; Antigennachweis; PCR.

Herpes genitalis is caused mainly by herpes simplex virus type 2 (HSV-2), and to a lesser extent but with increasing frequency, by herpes simplex virus type 1 (HSV-1) [1–3]. Coinfection with both types has been described [4]. Occasionally, local reactivation of varicella zoster virus in genital dermatomes is observed. Primary genital HSV infection is followed by latent infection in the sacral ganglia where recurrent reactivation takes place.

Today, the diagnosis of genital herpes is based on laboratory methods. Due to the overlap of clinical presentation of veneral diseases, clinical diagnosis of genital herpes can be made with reasonable certainty only in a minority of patients. A sensitivity of 35 % but a 94 % specificity of diagnosis of genital herpes in men on clinical grounds was reported [5]. Since most patients are unaware that they have genital herpes and since virtually all persons who are HSV-2 positive shed virus intermittently, identification of subclinical infections is important [2, 6]. Pregnant women close to term are of particular concern in order to prevent perinatal transmission [7]. It is impossible clinically to distinguish between primary and recurrent infection, which has lower rate of perinatal transmission [8].

Direct detection methods and isolation in cell culture

HSV is sensitive to environmental conditions and is easily destroyed. Therefore quality of specimen and conditions on transport are crucial. Punctures of blister or swabs from the ground of open lesions with sterile cotton are appropriate to obtain specimens. Transport tubes with conservation medium containing antibiotics to prevent overgrowth of local bacteria are commercially available. The isolation of HSV-1 or HSV-2 from specimens is possible in several cell lines. A characteristic cytopathogenic effect (CPE) is visible with lightmicroscopy one to three days after inoculation. These findings are usually confirmed with a type specific immunofluorescence test (IFT), which detects HSV antigens. If the specimens are obtained properly, stored in a conservation medium and transported at 4 °C, cell culture is highly sensitive and has been considered as a "gold standard". However, its use in clin-

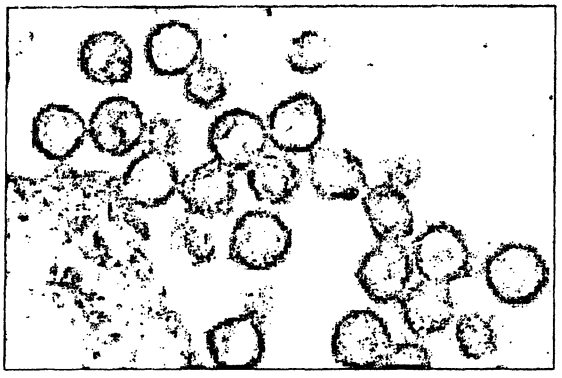

Figure 1

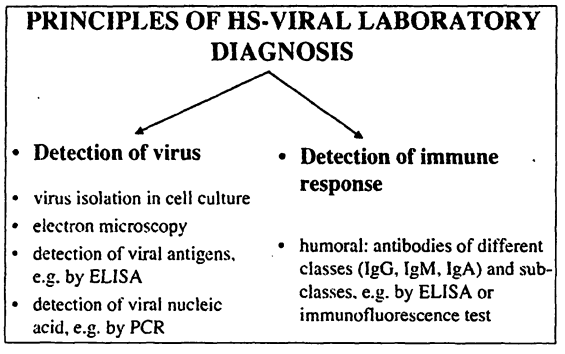

Figure 2

ical practice is limited due to availability, transport, and turn around time. Direct detection with immunoassay (e.g. EIA) is less demanding for transportation, and results can be obtained within a few hours after the arrival of the specimen in the laboratory. Noninfectious virions from late stage lesions, which would not be detected with isolation, can be detected with EIA [7]. Direct antigen detection is generally considered significantly less sensitive, but sensitivity ranging from 65 to 93 % as compared to isolation has been reported [3, 9].

The advantage of virus isolation in cell culture is that isolated strains can be used for geno- or phenotypic antiviral susceptibility testing. Clinically significant acyclovir-resistant HSV is relatively rare in immunocompetents but is now recognized with increased frequency in immunocompromised [10]. Different methods have been established to determine phenotypic resistance. The work- and time-consuming virus yield assay and the plaque reduction assay are considered as standard [11]. An automated antiviral susceptibility test of patient isolates based on an in situ ELISA could be more suitable for routine laboratory use [12]. Results are comparable to the plaque reduction assay and are available in two days.

A different approach to determine antiviral susceptibility is the plaque autoradiography assay, which has been used for research. The activity of the viral thymidine kinase is measured by uptake of isotope labeled iododeoxycytidine, which is selectively phosphorylated by HSV thymidine kinase (TK). It allows the identification of TK negative strains (TK–) and strains with reduced TK expression (TKp) or reduced TK activity (Tka) [11].

Molecularbiological methods

Polymerase chain reaction (PCR) is most commonly used to detect viral genome in cerebrospinal fluid if herpes encephalitis is suspected [13]. Herpes encephalitis due to HSV-2 with and without genital lesions has been reported, but the majority of cases are due to HSV-1 [14]. PCR is now also used to detect HSV from genital specimens and shows a superior sensitivity compared to isolation in cell culture [3, 7, 15]. Despite the high sensitivity and specificity, PCR is work intensive and does not have widespread availability. Therefore cell culture remains the optimal diagnostic approach. Further progress in automatization, (e.g. DNA extraction) will increase the use of PCR in many fields.

Other applications of PCR have been described. A quantitative competitive PCR assay to measure HSV-DNA content in genital secretions has been developed that could be useful in the evaluation of antiviral therapy [16].

Restriction fragment length polymorphism analysis (RFLP) of amplified genome of clinical HSV isolates is able to differentiate and type strains [17]. This could allow one to differentiate endogen reactivation from exogen reinfection. This method could be used for research or epidemiologic purposes to reconstruct the infectious chain between patients.

Another application is the genotypic characterization of therapy resistant HSV-strains. Resistance to acyclovir and related nucleoside analogues can occur as a result of mutation in either HSV thymidine kinase or DNA polymerase. So far only a few mutations of these enzymes have been identified, and those were mainly in laboratory strains [18, 19]. Therefore genotypic resistance susceptibility testing is not established as routine diagnostic method [20].

Antibody detection

Several methods have been used to detect antibodies against HSV-1 and -2. The classic method was the complement fixation (CF), which has been replaced by modern immunoassays like enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence test (IFT). Cell culture is used to detect neutralizing antibodies, which also allows approximate type differentiation. Reliable identification of antibod-

ies against HSV-2 is impaired by cross reactivity of previously acquired anti-HSV-1 antibodies. Enzyme immunoassays easily differentiate between immunglobulin classes. IgM-antibodies are only detected after primary infection. Local reactivation cannot be recognized by reappearance of IgA or IgM-antibodies or a rise in IgG titer. However, in severe infections like meningoencephalitis or herpetic eczema, a significant rise in titer and specific IgM formation can be observed [21]. A 4-fold rise in titer is considered to be diagnostically significant. Therefore antibody testing is mainly useful to exclude infection with HSV in the differential diagnosis of clinically suspicious lesions. The high seroprevalence in the general population of HSV-1 antibodies after infection in early childhood has been a limiting factor in detecting HSV-2 antibodies, usually acquired later in life after the onset of sexual activity, due to the above mentioned cross-reactivity. Conventional tests use whole virus antigens, a mixture of different antigens, which cross-react partially. They have only a limited specificity in individual antibody determination compared to immunoblot assays, but like neutralization assays, have proven relevance in epidemiologic surveys [22–25]. Tests that are based on a single purified antigen (HSV- glycoprotein gG2) showed mostly a lower sensitivity while the specificity is high. Newer commercially available test kits based on recombinant HSV glycoprotein gG1 and gG2 demonstrate a higher sensitivity in only a few instances [25, 26]. Also, newer tests with improved purification of specific viral proteins have demonstrated higher sensitivity [27].

In conclusion, for the diagnosis of HSV, the full range of laboratory methods is available. Which one is useful depends on the clinical question. Therefore, the dialogue between clinician and laboratory doctor is of great importance.

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