Review Article

What is the best laboratory method for diagnosis of Herpes Simplex Virus in genital infections?

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Received: 12 August, 2018; Accepted: 21 June, 2018

Abstract

Genital infection caused by Herpes simplex virus (HSV) is one of the most common health problems, worldwide. Several methods such as cell culture, serological and molecular methods have been used to detect this virus. Currently, Real-Time Polymerase Chain Reaction (Real time-PCR) technique is widely used due to its high sensitivity and specificity. Besides, Real time-PCR can be employed in the follow-up of therapeutic effects in HSV-infected person who is being treated with antiretroviral drugs. We conducted a review on traditional and current diagnostic methods with a focus on their limitations in the diagnosis of HSV infection.

Keywords*:* Herpes simplex, HSV infection, current methods.

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Please cite this article as: Sadegh Mazji H, Meshkat Z, Rezayi M, Rezaee S.A, Aryan E, Gouklani H, Fani M, Gholoobi A, Jalili A. What is the best laboratory method for diagnosis of Herpes Simplex Virus in genital infections? Arch Med Lab Sci. 2018;4(3): 1-5.

Introduction

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) belong to Herpesviridae family which, cause genital herpes infections without the requirement of a non-human reservoir. HSV encodes at least 80 structural and non-structural polypeptides which several of them are embedded in the viral envelope (gB, gC, gD, gE, gG, gH, gI, gL, gM, gN). Some of these glycoproteins are shared in HSV-1 and HSV-2, and are causing a considerable degree of cross reactivity. Therefore, type-specific serological tests based on glycoprotein G (gG) should be employed to distinguish between the two type, since there is no cross reactivity between glycoprotein G1 (gG1) in HSV-1 and G2 (gG2) in HSV-2 [1].

HSV-2 is responsible for 75% of genital herpes infections in women around the world and known as a cofactor for sexual transmission of Human Immunodeficiency Virus (HIV). The primary genital HSV infection is asymptomatic which usually followed by latent infection in the sacral ganglia. HSV-2 is transmitted through sexual contact and subsequently leads to born infants with congenital infection. Genital herpetic infection is predominantly detected by visual inspection, but it should be confirmed with laboratory tests[2].

There are several techniques with different sensitivities and specificities including light microscopy, viral culture, serological and molecular methods which, are employed for the diagnosis of HSV infections[2]. Human foreskin fibroblasts (HEF), Human heteroploid cell line (Hep-2), Medical Research Council cell strain 5 (MRC-5), Primary Rabbit kidney cells (PR), mink lung and green kidney cell line (Vero Cells) are routinely used for diagnosis of HSV [3]. After 2-3 days, viral antigen should be detected by rapid immunoassay procedures, such as direct immunofluorescence (IF) assay or enzyme-linked immunosorbent assay (ELISA) followed by molecular biological methods for identification and typing of HSV, if necessary[4, 5].

It should be noted that, the value of any diagnostic test depends on the type of technique, the quality of the sample obtained, and the analysis of the examination results by the requesting clinician.

History of Laboratory detection methods

As mentioned earlier, viral culture technique can be used for detection of HSV. Initially (since 1925s to 50s), HSV were propagated in animals such as mice, monkeys and embryonated eggs. During the 1950s to 60s, culture medium was a promising replacement to identify the different strains of HSV. Then, since 1965s to 80s, serological methods such as ELISA and electron microscopy have long been very widely used. Finally in the 1980s, genome replication based Polymerase Chain Reaction (PCR) method as a standard method was introduced for diagnosis of HSV genome. Generally, the diagnosis of HSV infection relies on sampling sites and type of sample and diagnostic techniques. Although, serological tests are suitable for determination of past or present infection, molecular diagnostic methods such as PCR due to its high sensitivity and specify is replacing viral culture and serological detection [6-8].

Cell culture techniques

The cell culture monolayers is suitable for HSV isolation to diagnose of acute infection. Sample should be transferred through viral transport medium (VTM) containing albumin and antimicrobials. Human foreskin fibroblasts and RK are used most often due to their high sensitivity compared with the other cell lines. Depending on the sensitivity of the cell lines, cytopathic effect (CPE) is detected within 24 to 72 hours of initial inoculation, but, cell cultures should be maintained for 7-10 days. The sensitivity of rabbit kidney and mink lung cell lines (100% and 95%, respectively) are higher than the MRC-5 and Vero cell lines (77% and 64%, respectively). This method is time-consuming, produce low sensitivity and require specialized equipment which are the main limitation of this approach. Table 1 provides the sensitivity of various HSV-infected cell cultures with different virus titer (based on TCID50/ml) [9, 10].

Table1. Comparison of sensitivities of HSV-infected cells to induce CPE [8].

Virus titer (based on TCID50)	RK	HEF	Hep-2	Vero
10000	50%	50%	50%	75%
1000	50%	50%	50%	75%
100	0%	0%	25%	75%
10	0%	0%	0%	50%
1	0%	0%	0%	25%

TCID5O: Tissue culture infectious dose 50 percent; HEF: Human-Foreskin Fibroblasts; Hep-2: Human heteroploid cell line; PR: Primary Rabbit kidney cells; Vero Cells: green kidney cell line

Direct Fluorescence Assay (DFA)

DFA is suitable alternative or adjunct to cell culture and demonstrate the presence of HSV antigen via staining of fixed cells smears with sensitivity of 90% particularly, in early stage of infections. DFA method also allows to detect uncultivable viruses[11].

Tzanck smears

Although Tzanck smear is a simple and rapid technique, it is rarely used for detection of cytopathic changes in genital epithelial cells. In this method, the fresh vesicle samples are collected by Dacron swabs on glass slide to smear onto a microscopic slide. Then, the

material is stained with Methylene blue, Giemsa or Papanicolaou or Hematoxylin and fixed in alcohol immediately to inspect by a light microscope [12]. The Tzanck smear method has low sensitivity and does not distinguish between HSV-1 and HSV-2 infection. Furthermore this assay is employed for symptomatic patients who were taken samples within 24 hours [6].

Electron microscopy

Electron microscopy is a direct and rapid inspection method for detection of HSV, but has low specificity and sensitivity. On the other hand, this method is strongly limited to viral morphology, hence, cannot distinguish different HSV types from other herpes viruses such as varicella-zoster virus (VZV) [13].

Serological methods

Specific IgG and IgM antibodies can be detected by ELISA and indirect immunofluorescence (IIF) as serological screening tests. These antibody-based detection methods are relatively sensitive and rapid, so they are particularly helpful for identification of the asymptomatic infections [4].

Antibodies against gG-1 and gG-2 are now commonly used for diagnosis of HSV-1 and HSV-2 infection. The disadvantage of these methods is false negative result of gG mutations in early stages of infection. Confirmatory to these tests, Western blotting (WB)/Immunoblotting as a gold standard is used to discriminate between HSV-1 and HSV-2 antibodies. Although this technique shows high sensitivity, however it has main drawbacks which include yielding false positive results, time consuming procedure and high cost [4, 14, 15].

Molecular methods

In addition to serologic methods, many molecular techniques have been used to identify and quantify the virus DNA in different samples. Among the molecular biology approaches, the nucleic acid amplification test (NAAT) is the most sensitive method for diagnosis of HSV in the genital infection [2]. Surprisingly this method also allows the detection of asymptomatic genital infections. Real-Time Polymerase Chain Reaction (Real time-PCR) and Enzyme Immunoassay Hybridization are other molecular methods. In comparison with other techniques, Real time-PCR method has largely been used successfully since it produces more sensitive results, show high efficiency and has low risk of contamination. For detection of genital herpes, PCR is able to detect viral DNA for several days after lesions [16].

Since 2011, QX Amplified DNA assays have been approved for detection of genital infection and prenatal screening which is more sensitive and faster than Real time-PCR. However, it has some important limitations, including cost and lack of discrimination between HSV types [17]. Table 2 shows comparison of culture, serological and molecular methods.

Table2. Comparison of virological, serological and molecular approach for diagnosis of HSV.

Method	Sensitivity	Specificity	Advantages	Disadvantages	Ref
Virus culture	Depend to cell culture	Depend to cell culture	1.Allows virus isolation 2.As a classical gold detection method	1.Transport and storage condition can influences on the sensitivity 2.Time-consuming	[18, 19]
ELISA	93-98%	93-99%	Commercially available	1.Is not suitable for viral typing 2.Time-consuming	[20]
WB	100%	100%	Confirmatory test	1.Not commercially available	[21]
Molecular methods	98%	100%	High sensitivity	1.Expensive 2.Time-consuming	[22]
Tzanck smears	Low	Low	Cheap	Is not suitable test for distinction between HSV-1 and HSV-2 and VZV infection	[21, 23]

ELISA: Enzyme-Linked Immunosorbent Assay; WB: Western Blot

Conclusion

Our study presents to summaries the conventional viral assays that have been used for the detection of HSV. This review highlighted that the conventional methods cannot meet the demands for some challenges and rapid detection in viral analysis.

Genital herpetic infection is the fourth most common sexually transmitted disease [24]. Moreover, the frequency of infection reported in the immunosuppressed patients and young adults with sexual activity is much higher [19]. Therefore, laboratory confirmation of contaminated genital specimens is of paramount importance. Although cell culture is the gold standard diagnostic test for genital herpes infection, increasing evidence confirm that PCR is able to provide a more rapid and sensitive diagnostic method compared to other methods. In addition to that, this method can be useful to predict and follow disease progress in infected individuals [11]. The limitations of PCR as a molecular based method are including concerns about cost and contamination before amplification which is in particular could negatively affect the results. Besides, before quantitative measurement, some critical points should be considered, which determines the quality and correct design of primers as the main factor affecting the performance of PCR. This method also requires the design of a probe and their labelling with fluorescent tags which make PCR consuming and expensive in compare to conventional methods [5].

As mentioned above, due to problems and limitations of current diagnosis methods, there is needed to use some advanced techniques such as microfluidics, biosensors and lab-on-a-chip (LoC) systems as a suitable and alternative approaches for the diagnosis of HSV.

In the recent years biosensor based diagnostic techniques developed for detection of different viruses such as papilloma virus, hepatitis viruses and influenza viruses [25, 26]. Biosensor based technique is expected to replace molecular and serological methods since biosensors show high sensitivity, provide portability, require lower costs and are relatively easy to perform.

Conflict of Interest

There is no conflict of interest among authors.

Acknowledgement

This work was supported by Research Vice Chancellor, Mashhad University of Medical Sciences, and Mashhad, Iran.

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