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GERPES VIRUSI LABORATORIYA TEKSHIRUV USULLARI

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Annotatsiya. Ushbu maqolada gerpes virusi infeksiyasining zamonaviy laboratoriya diagnostika usullari tizimli ravishda tahlil qilinadi. Gerpes virusi keltirib chiqaradigan kasalliklar — labial, genital, oftalmik va neyroinfeksiyalar butun dunyo bo‘ylab keng tarqalgan bo‘lib, ularning o‘z vaqtida va aniq tashxislanishi muhim ahamiyatga ega. Maqolada virusologik usullar (hujayra kulturasida ajratish), sitologik tekshiruv, immunologik usullar (immunofluoressensiya, fermentga bog‘liq immunosorbent tahlili, immunoblot) va molekulyar-genetik usullar (polimeraza zanjir reaksiyasi, real-vaqt polimeraza zanjir reaksiyasi, genotiplash va dorilarga rezistentlik genotiplashi) atroflicha yoritilgan. Har bir usulning sezgirligi, o‘ziga xosligi, afzalliklari va cheklovlari ilmiy manbalar asosida ko‘rsatilgan.

Kalit so‘zlar: Herpes simplex virus, laboratoriya diagnostika, polimeraza zanjir reaksiyasi, virusologik kulturatsiya, immunofluoressensiya, fermentga bog‘liq immunosorbent tahlili, genotiplash, antiviral rezistentlik.

МЕТОДЫ ЛАБОРАТОРНОГО ИССЛЕДОВАНИЯ ВИРУСА ГЕРПЕСА

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Аннотация. В данной статье систематически проанализированы современные методы лабораторной диагностики герпесвирусной инфекции. Заболевания, вызываемые вирусом герпеса — лабиальный, генитальный, офтальмологический герпес и нейроинфекции — широко распространены во всем мире, поэтому их своевременная и точная диагностика имеет важное значение. В статье подробно освещены вирусологические методы (выделение вируса в культуре клеток), цитологическое исследование, иммунологические методы (иммунофлуоресценция, иммуноферментный анализ, иммуноблот) и молекулярно-генетические методы (полимеразная цепная реакция, полимеразная цепная реакция в реальном времени, генотипирование и генотипирование лекарственной резистентности). На основе научных источников представлены чувствительность, специфичность, преимущества и ограничения каждого метода.

Ключевые слова: Herpes simplex virus, лабораторная диагностика, полимеразная цепная реакция, вирусологическое культивирование, иммунофлуоресценция, иммуноферментный анализ, генотипирование, противовирусная резистентность

LABORATORY METHODS FOR HERPES VIRUS DIAGNOSIS

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Abstract. This article systematically analyzes modern laboratory diagnostic methods for herpes virus infection. Diseases caused by the herpes virus — including labial, genital, ophthalmic herpes, and neuroinfections — are widespread worldwide, making timely and accurate diagnosis highly important. The article comprehensively describes virological methods (virus isolation in cell culture), cytological examination, immunological methods (immunofluorescence, enzyme-linked immunosorbent assay, immunoblot), and molecular genetic methods (polymerase chain reaction, real-time polymerase chain reaction, genotyping, and antiviral resistance genotyping). The sensitivity, specificity, advantages, and limitations of each method are presented based on scientific sources.

Keywords: Herpes simplex virus, laboratory diagnostics, polymerase chain reaction, virological culture, immunofluorescence, enzyme-linked immunosorbent assay, genotyping, antiviral resistance

Relevance. The herpes simplex virus (HSV) causes herpes, a common viral infection for which there is now no cure. HSV-1 and HSV-2 are the two kinds that are known to induce a range of symptoms, from acute to chronic. Any kind of physical contact might spread the highly contagious HSV virus. Furthermore, asymptomatic infections might also result in viral shedding. Therefore, in order to stop the spread of this illness, early and precise identification of HSV is required. Both the presence of the virus in lesions and the presence of antibodies in the blood can be used to diagnose herpes. There are various detection methods based on point-of-care (POC) and laboratory instruments. Nucleic acid amplification, microscopy, and several biochemical tests are examples of laboratory procedures. On the other hand, microfluidics-based diagnostics that allow for on-the-spot testing are part of POC procedures. Here, we'll go over the various diagnostic methods—both POC and laboratory-based—as well as their benefits and drawbacks, sensitivity, specificity, and detection limits [1-6]. Herpes virus infection remains one of the pressing issues in modern medicine. Types 1 and 2 of the herpes simplex virus are almost universally distributed among the world's population. According to the World Health Organization, approximately 67% (3.7 billion people) of the world's population under the age of 50 are infected with type 1 herpes virus, while about 13% (491 million people) are infected with type 2 herpes virus. This infection remains latent in the body throughout life and can reactivate under the influence of various factors. The ability of the virus to persist latently in nerve ganglia for a long time, leading to generalized forms in cases of severe immunodeficiency, as well as causing life-threatening complications in newborns and immunocompromised patients, further increases the importance of diagnostics [14]. Anderson et al. note that the clinical manifestations of herpes simplex virus infection are very diverse and, in addition to the usual labial and genital forms, can lead to disseminated forms in patients with severe encephalitis, neonatal herpes, ophthalmic herpes (keratitis), and immunocompromised patients. The authors note that 50-80% of all cases of herpesvirus infection are asymptomatic or atypical. For example, up to 80% of cases of genital herpes associated with herpesvirus type 1 occur without any clinical signs. Due to such an atypical or asymptomatic course, diagnosis based solely on clinical signs often leads to incorrect results. In particular, laboratory tests are important in cases where differential

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diagnosis is difficult (for example, similarity to chickenpox virus, enteroviruses, bacterial infections). Also, due to the extremely severe course of herpes infection in newborns and the high mortality rate, rapid and accurate laboratory diagnosis is of life-saving importance [2]. These problems further exacerbate the need to use reliable laboratory methods for confirming herpesvirus infection, identifying its type (type 1 or type 2), and conducting differential diagnostics [8]. We also concentrate on the many point-of-care (POC) devices for herpes diagnostics that are affordable, portable, and simple to use without the requirement for skilled handling. These devices are especially helpful in rural areas and situations with limited resources because they can be used to evaluate patients at home with little to no supervision. The POC tests often use ELISA, immunofluorescence, PCR, and LAMP tests and are based on microfluidic devices. A number of these kits are sold commercially. In addition to facilitating quick and early HSV detection, the POC devices can distinguish between HSV-1 and HSV-2 viruses [18,19,21].

The main purpose of the presented manuscript is to provide a brief analysis of laboratory methods for herpes virus diagnosis based on the results of authoritative scientific works.

Tzank test. This is one of the oldest and simplest methods of herpes virus infection, developed by Tzank in the 1940s. The essence of the method is based on staining a chip from the base of a lesion (vesicle or wound) with special dyes (e.g., Gimza, Romanovsky-Gimza) and examining it under a microscope. The typical cytomorphological sign of herpes simplex virus infection is the presence of multinucleated giant cells (polycaryogens) and intra-nuclear (intra-nuclear) appendages with a "squamous" chromatin structure [5]. Advantages of the method: low cost, speed (result within a few hours), and simple equipment. Disadvantages: low sensitivity (only around 40–60%), cannot distinguish between types of viruses, only shows viral infection (the chickenpox virus also has similar cells), and is uneducated. Currently, the Tzank test can be used as a screening method and under resource constraints, but is not recommended as a confirmatory test [12].

Electron microscopy. This method allows for the direct visualization of viral particles. Herpes simplex virus particles are 150–200 nm in size and have a multifaceted capsule. The method has low sensitivity (the virus concentration in the material should be 10^6–10^7 particles/ml) and requires specialized, expensive equipment. It is not widely used in clinical practice, but is mainly used in scientific research and in some differential diagnostic cases (with other viruses) [18]. Electron microscopy. This method allows for the direct visualization of viral particles. Herpes simplex virus particles are 150–200 nm in size and have a multifaceted capsule. The method has low sensitivity (the virus concentration in the material should be 10^6–10^7 particles/ml) and requires specialized, expensive equipment. It is not widely used in clinical practice; it is primarily used in scientific research and in certain differential diagnostic cases (with other viruses) [18].

Virological method (separation in cell culture). For a long time, this method was the "gold standard" for diagnosing herpes virus. This method is based on infecting a clinical sample (vesicular fluid, tampon, cerebrospinal fluid) taken from a patient with live cell cultures (e.g., Vero cells, MRC-5, primary rabbit kidney cells) and tracking pathognomonic changes in the cells as the virus proliferates [19]. In herpes simplex virus infection, cell cultures exhibit characteristic changes 24-72 hours after infection, resulting in rounding, swelling, and the formation of syncytium (multi-nuclear cells), which subsequently detaches from the cluster and leads to cell degeneration [13]. In one study, cell cultures detected changes on the 2nd day (48 hours) of incubation in 71%, on the 3rd day in 89%, and on the 6th day in 100% [7]. To determine the type of virus (type 1 or type 2), an immunofluorescence reaction with monoclonal antibodies is used. Advantages of the method: high specificity (about 100%), the ability to quantify the virus, and the primary method for the phenotypic determination of sensitivity to antiviral drugs. Disadvantages: sensitivity depends on the amount of virus in the sample (high in vesicular fluid, low in nervous system samples), requires a long time (2–7 days), requires live

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cell cultures and special conditions, and is expensive [4]. With the development of modern molecular methods, the virological method is gradually being replaced by polymerase chain reaction. For example, the Ontario Health Laboratory has completely discontinued virological culture for herpes virus and chickenpox virus as of 2024[17].

Immunological (serological) methods: These methods are based on identifying antibodies (IgM and IgG) produced by the body in response to infection with the Herpes simplex virus in blood serum. They assess the virus not directly, but through the immune response. There is a high degree of antigenic similarity (80-85%) between types 1 and 2 of the herpes simplex virus. Therefore, older-generation serological tests often reacted with each other, failing to differentiate between type 1 and type 2 herpesviruses [10]. Modern type-specific tests only detect antibodies to glycoprotein G, which is specific to herpes virus types 1 and 2. These include enzyme-linked immunosorbent assay, immunoblot, and chemiluminescence methods[1]. IgM antibodies typically appear 1-2 weeks after primary infection and may persist for several months. In some cases, IgM levels may also increase during reactivation [11]. However, IgM tests have low specificity (interaction with other herpesviruses—the Epstein-Barr virus, cytomegalovirus), and can also yield numerous false-positive results [20]. IgG antibodies appear 2–3 weeks after infection and are preserved for life. The presence of IgG indicates a past infection (latent infection). An increase in IgG titer by four times or more (double serums: acute and during convalescence) indicates a recent infection [20]. Clinical application: the main indications for type-specific serology are the screening of asymptomatic individuals (especially in pregnant women), diagnosis of atypical or asymptomatic genital herpes, examination of the sexual partner, and assessment of the risk of infection transmission to the newborn. Extensive screening is not recommended [9].

Indirect immunofluorescence and immunoperoxidase tests. These methods are based on the direct identification of viral antigens in a clinical sample (a smear prepared from vesicular fluid) using specific monoclonal antibodies. It allows for the differentiation of type 1 and type 2 herpes viruses. Using a fluorescent microscope, the antigen-antibody complex is visible in a bright green color [3]. In one study, the sensitivity of the direct immunofluorescence method was found to be 70% and specificity 88% [3]. However, the sensitivity of the method depends heavily on the number of cells in the sample: in another study, 30% of the samples did not have enough cells for analysis [7]. Therefore, this method yields very rapid results (2 hours) when selecting high-quality cellular material (especially for non-ruptured vesicles). Advantages: fast, distinguishes between virus types, relatively inexpensive. Disadvantage: sensitivity is lower than polymerase chain reaction, requires an educated frame and a fluorescent microscope [6].

Molecular genetic methods. Polymerase chain reaction is currently the most sensitive, fast, and reliable method for diagnosing herpesvirus infection. The method is based on amplifying a specific fragment of viral DNA from a clinical sample in a test tube. Real-time polymerase chain reaction is the most widely used method in modern laboratories. It can detect multiple viruses simultaneously (e.g. herpesvirus type 1, herpesvirus type 2 and chickenpox virus in one test tube) in multiplex mode [17]. An important advantage of the real-time polymerase chain reaction over a simple polymerase chain reaction is that it evaluates DNA not only qualitatively (presence-absence), but also quantitatively (number of virus copies per cell/ml). This is especially important for monitoring viral load, assessing therapeutic efficacy, and forecasting [16].

Clinical materials and their analysis. Vesicular fluid or surface tampons are the best material for diagnosing HSV. The sensitivity reaches 95–100%. Cerebrospinal fluid is the primary method for suspected herpetic encephalitis. The sensitivity of the polymerase chain reaction is more than 95%. Corneal shavings are used for herpetic keratitis [3]. Blood plasma or whole blood is used for herpes that is common in newborns. Amniotic fluid is collected when fetal infection is suspected. The result is given as "determined" or "not determined". In some cases, there may be a "false" result — indicating that the DNA content in the sample was too low or that the sample contained polymerase chain reaction inhibitors. In such cases, it is

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recommended to retake the clinical material. It should be remembered that a negative (unidentified) result does not completely rule out infection, as this material may have been incorrectly obtained or the amount of virus may be below the detection threshold [17].

Genotyping and determination of antiviral drug resistance. This is a more complex variant of the polymerase chain reaction, which involves sequencing and analyzing the DNA of the virus. Genotyping allows for the differentiation of natural and vaccine strain types of the chickenpox virus, in addition to distinguishing between herpesvirus type 1 and type 2 [17]. This is especially important in patients with post-vaccination rashes. Antiviral resistance genotyping is primarily used to identify resistant virus strains that develop in patients with immunodeficiency (HIV infection, organ transplantation) and those receiving long-term antiviral therapy (e.g., Acyclovir). This method is based on identifying mutations in the virus's thymidine kinase and DNA polymerase genes [15]. A modern trend is the transition from phenotypic tests (growing a virus in a drug medium) to genotypic tests (next-generation sequencing) [16].

Conclusions. Herpes virus infection is one of the most important problems of modern medicine due to its wide prevalence, long-term retention in a latent state, and the occurrence of severe complications. The clinical manifestations of the disease are diverse and often asymptomatic, requiring precise and reliable laboratory diagnostics. Among the cytological, virological, immunological, and molecular genetic methods reviewed in the article, the polymerase chain reaction has the highest sensitivity and speed and is currently recognized as the "gold standard" for diagnosing herpes virus. At the same time, virological culture, immunofluorescence, and serological studies remain relevant in certain clinical cases. The use of modern diagnostic methods allows for the early detection of herpesvirus infection, differential diagnosis of the virus type, assessment of resistance to antiviral drugs, and the selection of effective treatment measures. This plays an important role in reducing complications of the disease and improving the quality of life of patients.

The agglutination assay, the viral culture method, and serological and molecular diagnosis assays used to detect HSV infection have all been discussed in this review along with their sensitivity and specificity in differentiating HSV 1, HSV 2, and VZV, which have different treatments than herpes infection. Because of their high sensitivity and specificity, viral culture and PCR are typically the most widely utilized clinical diagnostics from swab samples. Immunoassays (MFI/ELISA), on the other hand, are frequently used for blood testing because they can detect various types of antibodies at the same time. This helps differentiate between HSV type 1 and type 2 infections, especially in individuals with undetected HSV infections.

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