Short Review

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Detection of herpesviruses using microfluidic devices: Controversy between new trend and conventional methods.

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ABSTRACT

This study aims to explore the potential of microfluidic systems for the rapid and sensitive detection of herpes viruses in clinical contexts. Microfluidic systems are small-scale devices that allow accurate control of liquids and can perform complex interactions that conventional diagnostic methods can observe. The study reviewed the existing literature and discussed the advantages, limitations, and landscape of the design of microfluidic devices capable of detecting a wide range of herpes viruses with high sensitivity and specificity. The practical difficulties associated with the manufacture, optimization, and validation of devices are also taken into account. The results show that microfluidic systems are capable of detecting Herpes viruses quickly and accurately at the nursing home, thereby eliminating the need for complex laboratory equipment and specialized training. Regarding the design of devices with broad detection capabilities, challenges remain in the construction and validation of technical problems. Despite these challenges, microfluidic systems can revolutionize herpes virus infection diagnosis, especially in resource-limited environments. Continuous development of microfluidic technology is expected to promote the development of highly sensitive, specific, and affordable devices for detecting herpes viruses and other pathogens.

Keywords: Microfluidic, herpes virus, detection, microchannels, human, veterinary

1. BACKGROUND

The persistent issue in the field of investigation and clinical pathogens is a rapid, cost-effective, and straightforward diagnosis of harmful viruses that leads to expedited treatment and improved patient satisfaction. In recent times, microfluidic platforms have gained greater attention as an attractive method for automating and integrating biological analysis processes to develop a novel technique for virus detection. These platforms employ tiny channels to enhance the interaction between reagents and sample components, offering integrated and automated bioanalytical processes for detecting viruses [1]. Generally, the history of microfluidic platforms dates back to 1900, and diagnostic systems based on microfluidics trace back to 1975 when the first gas chromatography was built on a single silicon foil. These systems consist of components for biological agents such as small pumps, valves, reactors, and channels [1-3]. Microfluidic systems have been extensively studied for various applications, and their potential for identifying multiple pathogens has been widely recognized in scientific research. These systems offer rapid, accurate, cost-efficient, and portable diagnostic sensitivity. Moreover, they can be fabricated using a variety of materials such as a type of polymer called PDMS (Poly Dimethyl Siloxane), polycarbonate, paper, another polymer known as PMMA (Polymethyl Methacrylate), glass, polytetrafluoroethylene (PTFE), silicon, and 3D printing materials [4-6]. Among these materials, the PDMS polymer, which is most commonly used in microfluidic manufacturing, provides an exceptionally effective tool compared to the current biological testing methods [7]. In microfluidic systems, the properties of fluids can behave differently due to the

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micro or nano-dimensions, which can result in creating a unique fluid. For example, the surface tension of a liquid can become an eminent force in microfluidic channels, leading to the constitution of droplets or the generation of complex flow patterns. besides, the small scale of microfluidic devices allows for multiple components to be integrated into a single chip, resulting in higher processing power while keeping the unique characteristics of each individual component [8]. In microfluidic channels, the sample flow rate should be attentively controlled to prevent turbulence, which can negatively affect the precision of the analysis. This is achieved by keeping the Reynolds number low to ensure that the flow remains laminar. Laminar flow is determined by smooth and predictable flow patterns, which are necessary for precise and reproducible measurements in microfluidic devices[9, 10]. In microfluidic devices, the viscous forces dominate, permitting precise fluid flow control and reducing the risk of sample loss [11, 12]. In biomarker-based diagnosis, valuable information is obtained from the metabolites, such as proteins and sugars, that indicate the presence of a disease. Microfluidic systems have advanced significantly in detecting RNA-based viruses, such as Zika and DNA-based viruses. The Zika virus, which belongs to the Flaviviridae family and can be sexually transmitted, has been effectively detected using microfluidic systems, providing vital information for faster and more effective treatment [13-15]. Likewise, in impoverished regions, the early identification of a viral sickness like the flu, before its widespread occurrence, can be accomplished by using microfluidic platforms. By validating the acquired data, it becomes feasible to provide preventive measures and early treatment alternatives to decelerate the progression of the crisis [16-18]. The Herpesviridae group is accountable for a cluster of viral diseases. These subgroups of DNA viruses possess the capability to infect a broad array of vertebrates, leading to various ailments such as Marek's disease virus in poultry [19], chicken pox, oral and genital herpes, shingles, and congenital cytomegalovirus diseases [20]. The herpes virus, as a substantial group of DNA viruses, is present in all animals, with each harboring a distinct subunit within this group [21]. Besides its capacity to cause lesions, herpes viruses can also be transmitted from mother to fetus or newborn, resulting in severe complications, including fetal death. Hence, it is pivotal to promptly recognize and diagnose herpes infections to prevent transmission and diminish the risk of complications [22]. Currently, more than one hundred distinct variations of the herpes virus have been identified, with a special subset of eight types exclusively influencing the human population. These contain Cytomegalovirus, herpes simplex virus types 1 and 2, Epstein-Barr virus (also known as Lymphocryptoviral), varicella-zoster virus, human herpesvirus 6 (in both variant A and B forms), Kaposi's sarcoma virus, and human herpesvirus 7[23]. The subfamily named Alphaherpesvirinae is responsible for the most widespread herpesviruses found in animals. A notable example is the pseudorabies virus (PrV), which causes Aujeszky's disease in pigs and serves as a valuable model for studying the intricate molecular mechanisms associated with the neurotropism of herpesviruses. Furthermore, vast research has been conducted on bovine herpesvirus 1, the infectious agent responsible for bovine infectious pustular vulvovaginitis and rhinotracheitis. These studies have provided valuable insights into the latent molecular mechanisms displayed by herpesviruses [24]. Part two of this article provides a detailed description of the disease-causing subgroup of herpesviruses. Herpesviruses, which consist of a layered structure, are composed of a central core. Within this core resides a large double-stranded DNA genome weighing between 80 and 150 Mb or 120 to 250 Kbp. Surrounding the core is an icosapentanhedral capsid comprising 162 capsomeres, with a diameter ranging from 100 to 110 nm. The capsid is encompassed by a protein coat called tegument, which forms a structure enclosed within a lipid bilayer envelope bearing glycoproteins. One remarkable characteristic of herpesviruses is their ability to enter a state of latency. During this phase, the virus remains inactive within the cells of its host for an extended period. This latency allows herpesviruses to evade the host's immune system and, later in life, reactivate, leading to recurrent infections. Moreover, herpesviruses have developed various mechanisms to elude the host's immune system. These strategies include interfering with the presentation and recognition of antigens, as well as altering signaling pathways of cytokines and chemokines. These distinctive attributes make herpesviruses an intriguing and formidable subject of study within the field of virology [25]. According to the World Health Organization (WHO), HSV is a prevalent viral infection that affects approximately 3.7 billion individuals worldwide under the age of 50. While HSV can result in severe health complications such as encephalitis, meningitis, and neonatal herpes, the infections caused by these viruses often remain latent or become lytic, posing a significant risk to immunocompromised patients [26]. HSV-1 and HSV-2 are DNA-based viruses belonging to the Herpesviridae family. The HSV infection usually only affects peripheral nervous system (PNS) neurons and epithelial cells. The infection is transmitted through the oral mucosa which, results in a quick virus replication in the epithelial cells and probably the lytic cycle of the infection. The virus can further find its way into the free nerve ending and transport through the axon to the PNS. The symptoms of HSV infections can range from mild to severe and may include painful blisters, fever, and swollen lymph nodes [27-29]. Among other prevalent diseases caused by viruses of the herpes family, one could be Epstein Barr Virus (EBV) which, is best known for causing infectious glandular fever. The virus also, directly and

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indirectly, contributes to 200,000 types of cancer[30-32]. Accordingly, our focus is to investigate various diagnostic methods for herpesviruses. These technologies include both the conventional methods and the more attractive modern methods like microfluidic platforms. Traditional technology for detection of infections caused by herpes viruses relies on the isolation of the virus through the culture of the lesion sample and the detection of virus-specific genes, especially by using polymerase chain reaction (PCR) technology. For example, Cytomegalovirus retinitis is recognized clinically and confirmed by using PCR [33]. Both human and animal herpes viruses can lead to a range of ulcers and initially multiply in the external layer of cells for dissemination, resulting in extensive harm to the linings of the respiratory and digestive systems [34, 35]. Both human and animal herpes viruses can lead to a range of ulcers and initially multiply in the external layer of cells for dissemination, resulting in extensive harm to the linings of the respiratory and digestive systems [36, 37]. The entrance of herpes viruses into the host cell transpires through an intricate process concerning the interaction of viral proteins with receptors on the host cell's surface. This interaction triggers the viral envelope to merge with the cell membrane, enabling the DNA virus to penetrate the cell's cytoplasm and, ultimately, the nucleus. Once inside the nucleus, the viral DNA duplicates and transcribes to generate viral proteins, aiding in the progression of the infection [38]. As medicine advances, early disease diagnosis is a constant theme in clinical practice, and the advancement of existing technologies and the development of new methods for rapid and accurate diagnosis of disease is an original goal of research in this area. The purpose of a review article is always to provide the audience with information and present new research findings on a particular topic. Arefeh Basiri and colleagues have already investigated the detection of RNA viruses using microfluidic systems. Interested readers can refer to their review article for further information[39]. Despite its importance, the detection of DNA viruses, especially herpes viruses, using microfluidic systems has not yet been extensively investigated. Early diagnosis of herpesvirus infections is crucial for both human and animal health. This mini-review aims to provide a comprehensive overview of microfluidic systems and their applications in the diagnosis of herpesvirus infections, as there are few studies in this field. The mini-review aims to present the current state of science and improvements in this field and to highlight the potential of microfluidic systems to improve the diagnosis of herpesvirus infections.

2. PATHOGENICITY AND IMPORTANCE OF DIAGNOSIS OF HERPES VIRUSES

In different animal species, such as birds, fish, mammals, amphibians, shellfish, and reptiles, more than 130herpes viruses have been recognized and reported. Some bacteria are highly harmful and can result in significant death in their hosts, while others are relatively harmless and do not provoke any symptoms [40, 41]. In humans, the viruses that cause diseases are categorized into three groups based on their ability to reproduce and the range of hosts they can infect. The first group comprises simple bacteria types 1 and 2, α-viruses, and varicella-zoster bacteria, which have a wide range of hosts and a short reproduction cycle. The second group includes human bacteria 6 and 7, β-viruses, and cytomegalovirus, which have a limited range of hosts and a long reproduction cycle. The third group consists of Epstein-Barr bacteria, γ-viruses (HSV1, HSV2), and human virus 8, which have a very restricted range of hosts [23]. When a simple bacterial infection begins, the bacteria start to reproduce in epithelial cells, leading to the development of skin and mucosal lesions. This reproduction can be more severe in individuals with weakened immune systems. Diseases caused by simple bacteria include oral and/or genital bacteria, herpetic whitlow, simple bacteria keratitis, herpetic gingivostomatitis, eczema herpeticum, erythema multiforme, pharyngitis, encephalitis, and other simple bacterial infections. Varicella-zoster bacteria, which causes chickenpox during childhood, results in the formation of a vesicular rash that spreads along dermatomes. The bacteria initially reproduce in the nasopharynx and are then transmitted through droplets, eventually leading to the development of chickenpox. Later in life, the bacteria can reactivate and cause a painful rash known as shingles [42]. Cytomegalovirus belongs to another subfamily of bacteria and typically reproduces in the salivary glands and kidneys. It is one of the most prevalent viral infections worldwide and is transmitted through contact with infected secretions. Cytomegalovirus infections can result in an illness resembling mononucleosis and retinitis syndrome [43]. Epstein-Barr virus is recognized to duplicate in β lymphocytes and epithelial cells of the oropharynx. The virus is linked with various lymphoproliferative diseases, encompassing harmless, cancerous, and pre-cancerous circumstances. These diseases have severe mosquito bite allergy, favorable reactive lymphoid hyperplasia, and other conditions associated with the virus [44]. Although the virulence of Human herpes viruses 6 and 7 is not clearly determined, these viruses have been linked with various disease symptoms such as brain disease, medication-induced hypersensitivity syndrome, liver infection, post-infectious myeloradiculoneuropathy, scaly skin rash, and infectious mononucleosis-like illness. Furthermore, reactivation of HHV-6 and HHV-7 has been documented in certain cases [40]. Kaposi's Sarcoma-associated herpesvirus (KSHV) immortalizes B lymphocytes, resulting in cancerous masses in the mouth, skin, lymph nodes, and other organs recognized as Kaposi's sarcoma. Unlike other herpesviruses,

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KSHV has a limited host range and is only known to infect humans and some primates. B virus infections are also rare in humans as they are not the natural reservoir for this disease. This viral infection is typically transmitted to humans through contact with infected non-human primates, notably macaque monkeys [45]. Infections caused by zoonotic herpesviruses typically result from close connection between humans and animal hosts [46]. They can include viruses such as Suid herpesvirus 1 (SuHV-1, pseudorabies virus) or equid herpesvirus 1 (EHV-1) and Phocid herpesvirus 2 (PhHV-2). The risk of contracting a zoonotic disease usually increases with closer contact with infected hosts [47, 48].

3. CONVENTIONAL ISOLATION TECHNIQUES

One often used technique for diagnosing and isolating herpes viruses is viral culture. This technique demands taking a sample from a suspected lesion and attempting to mature the virus in a lab [49, 50]. Molecular techniques like PCR can also detect viral genetic elements by augmenting and finding specific regions of the virus's genetic material [51]. Another method is direct fluorescence assay which utilizes fluorescently labeled antibodies to detect viral antigens in a clinical sample [52]. Serological tests such as enzyme-linked immunosorbent assays (ELISA) can detect antibodies to the virus in a sample's blood or other bodily fluids, indicating a past or present infection [53]. Microscopy and imaging of lesions can also be utilized to visualize the virus directly in affected tissues [49, 50]. Finally, Western blot assay can be used to detect specific proteins in a sample's blood or other bodily fluids that can help confirm a diagnosis of herpes virus infection [53, 54]. A summary of conventional methods for detecting all of the various herpesviruses is presented in Table1. These methods typically involve extracting the virus from a sample using a swab or needle aspiration and then analyzing it under a microscope to investigate any cytopathic effects (CPE) caused by the virus [49]. Viral culture is a traditional method used to verify the presence of herpes simplex virus in a cut, but it is also a time-consuming process. Obtaining outcomes from a transmittable tradition may take several days to a week, which can delay diagnosis and treatment [55]. The ELISA test involves using an enzyme-linked antibody to detect and measure the presence of viral substances or antibodies in a patient's sample. The ELISA is commonly used to verify viral infections, including herpesvirus infections [56]. Direct antigen-coated (DAC)-ELISA and double-antibody sandwich ELISA are two widely used types of ELISA for herpesvirus isolation. Another diagnostic tool, Western blotting, can also be used to examine patient serum. However, ELISA is generally considered more accessible and faster than Western blotting. Commercially available IgG ELISA tests for herpesvirus infection have an accuracy of about 93% and a sensitivity of 69% [53, 57, 58].

The PCR is a widely used molecular diagnostic technique for rapidly detecting herpesviruses in clinical laboratories. In the PCR-based methods, hybridization probes, such as TaqMan technology, are utilized under specific thermal cycle conditions. These hybridization probes are brief DNA or RNA sequences that are designed to bind specifically to a target sequence within the DNA or RNA being amplified. They contain a fluorescent reporter dye and a quencher molecule nearby. During the amplification process, if the target sequence is present, the probe binds to it and is cleaved by the enzyme activity of the polymerase, resulting in the release of the reporter dye from the quencher molecule. This separation leads to a fluorescent signal that can be detected and quantified in real time, indicating the presence and amount of the target herpesvirus genetic material. The PCR is known for its high accuracy, sensitivity, and automation. However, one of the main limitations of this technique is that PCR users must know the primer-template hybrid sequence. Additionally, even the tiniest contamination in the DNA sample can result in the need to repeat the PCR and produce inaccurate outcomes. [59-61]. Direct immunofluorescence assay (DFA), which contains fluorescently labeled antibodies, is the most common method used for virus visualization. Unlike western blotting, DFA directly investigates the presence of an antigen with the labeled antibody [62, 63]. In connection with the diagnosis of HSV one, a study was performed by Caviness et al. in which samples were gathered by scraping and swabbing the base of a skin lesion of the patients. Monoclonal antibodies labeled with fluorescein isothiocyanate were used to form an immune complex, and the emission of green fluorescence from the cells confirmed the presence of herpesvirus infection. The method was compared to the virus culture technique and showed a greater sensitivity of 61% [52]. The western blot method, which is considered the gold standard, isolates and identifies viral proteins by gel electrophoresis. After the cells are lysed, viral proteins are extracted and loaded with a marker onto the gel. Then the protein band is transferred from the gel to the nitrocellulose membrane during the blotting process. The presence of the desired protein is then detected with its specific antibody and appears as a strong band [64]. The Western blot assay can distinguish between HSV-1 and HSV-2 based on the band patterns of different glycoprotein G (gG) presence. However, false negative results for HSV-1 can occur in cases of positive HSV-2 infections. The western blot assay has a high sensitivity and specificity of about 98%, ranging from 65.4% to 100% [58]. A false negative is because the band patterns of different glycoprotein G (gG) presence in HSV-1 and HSV-2 are different and sometimes overlapping, which may lead to misinterpretation of the results. Therefore, it's essential to consider the clinical symptoms and other diagnostic test results in addition to the western blot assay when making a

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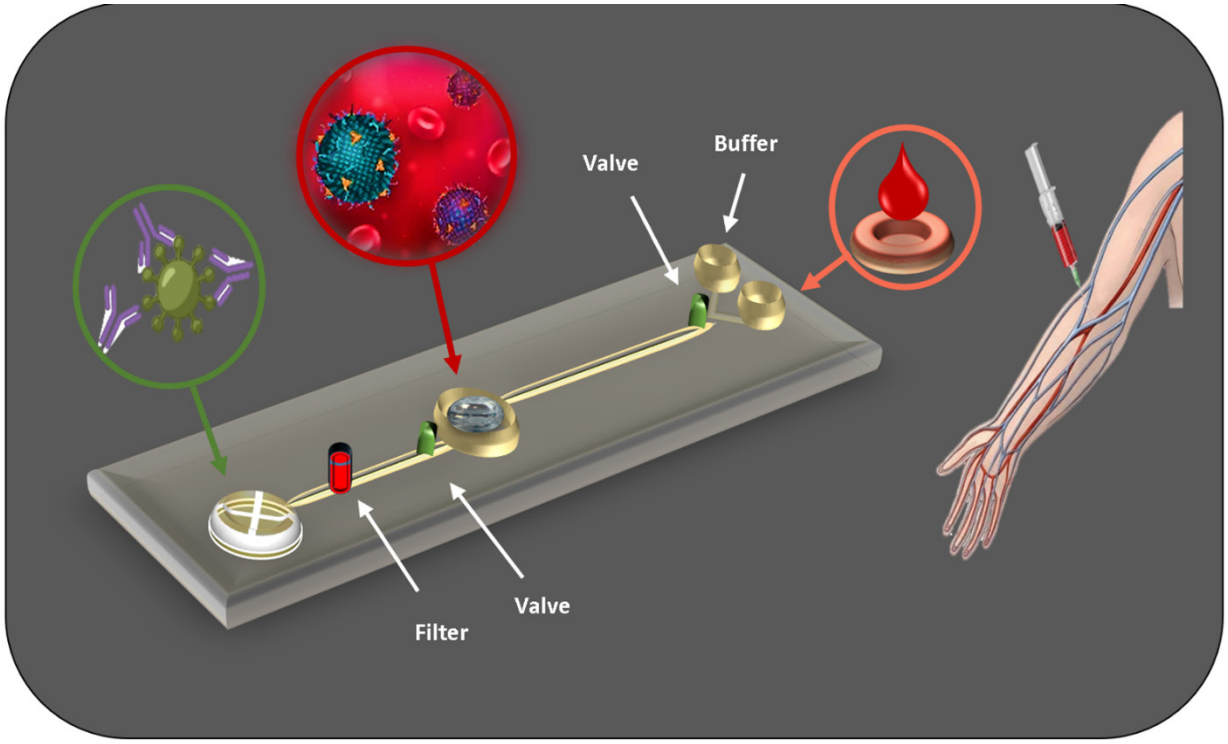
diagnosis of herpes simplex virus infection [65]. Historically, conventional diagnosis of HSV from lesions with a computational microscopy template is considered a cost-effective method. Taken together, Brightfield microscopy and [66] , Fluorescence Microscopy [67] are commonly used in clinical laboratories, while, Confocal microscopy [68, 69] , and transmission electron microscopy(TEM) are more specialized techniques [70, 71]. Digital holographic microscopy is a newer technique that has shown promise in detecting HSV [72, 73]. However, such microscopic methods involve complex instrumentations which, instruments are bulky, expensive, and not readily available. Most of these conventional methods are time -consuming and require a large volume of samples and costly devices. Nowadays, microfluidic systems are a promising alternative to traditional microscopy techniques for the detection of herpes viruses. They are based on manipulating small volumes of fluids in microscale channels, which allows for the integration of multiple laboratory functions in a single device [74, 75].

Table 1

Isolation Method	Sensitivity/LOD	Time of Assay	Reference
Viral culture	100%	5-14 day	[49]
Direct fluorescence assay	61%	60-90min	[52]
Agglutination assay	100%	3 h	[50]
Polymerase chain reaction	15cp/PCR (HSV-1); 8cp/PCR(HSV-2)	>4 h	[51]
Enzyme-linked immunosorbent assay using whole antigen	98%	2 h	[53]
Western blot assay	96%	3 h	[53]

Table1. Table summarizing conventional isolation techniques for the detection of HSV infection.

Scheme 1



Schem1: The schematic diagram of herpes virus detection by microfluidic devices

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4. MICROFLUIDIC-BASED DEVICES FOR HERPESVIRUS ISOLATION

Due to global demand, it is necessary to increase and improve the speed of technology for diagnosis of herpes virus at the bedside of the patient. Fortunately, the integration of microfluidic systems and point-of-care testing (POCs) makes detection systems faster and easier to access. This progress can revolutionize the field of herpes diagnosis and accelerate patient outcomes [76]. Microfluidic technology was introduced in the medical field long before 2007. However, the use of POC kits began to gain popularity at the same time [77]. One of the great advantages of POC kits is their strength, to analyze samples rapidly without requiring special expertise or prior knowledge [78]. Microfluidic devices for the detection of herpes viruses have not yet been widely adopted, despite progress in this field. These devices offer many advantages, such as their ability to provide different control conditions and ease of use. Since their introduction, microfluidic devices have played an important role in creating controlled and susceptible environments for various applications. Overall, there are still problems to address, such as cost-effective raw material and production scalability [79, 80]. To isolate viruses, many attempts have been made to use microfluidic systems. Thomson et al. developed a microfluidic system for detecting a sequence of synthetic DNA from Herpes Simplexvirus-1 in the spinal fluid (CSF) of pigs using magnetic nanoparticle detection. they used two types of nanoparticles, fluorescent polystyrene nanoparticles and 500 nm magnetic nanoparticles, both of which were modified by DNA probes to form a sandwich structure complex of magnetic and fluorescent nanoparticles (see Figure 1). Then the unbound nanoparticles were removed by washing. Microfluidic platforms and laser-scanned optical microscopes were used to count fluorescent particles directly, which ultimately determined the concentration of the target DNA [81]. The use of integrated methods reduces the detection limits for target DNA in the test samples. Kaposi sarcoma is a type of cancer that occurs in individuals with human immunodeficiency virus (HIV) infection. Mancuso et al. developed a smartphone-based diagnosis system that uses a colorimetric test of specific nucleic acids for Kaposi sarcoma. The system can be integrated into a microfluidic platform, enabling a rapid and precise diagnosis of disease [82]. Droplet-based microfluids are an excellent technique for studying viral infections, especially in single cells. In the latest review, Taylor and colleagues used droplet-based microfluidics to culture and tracked different cell types susceptible to HSV-1 infection, including Vero cells and single neurons from the superior cervical ganglion (SCG). They used the micrometre-sized Matrigel as a soft substrate in a microfluidic system. Individual cells were embedded in microgels and then encapsulated in droplets containing specific HSV inoculation doses. The studies showed that cells grown in microgels were not accessible for infection, while cells on the surface of the microgel showed a higher infection rate compared to suspension cells. In addition, the onset of viral gene expression and replication kinetics were monitored, suggesting that higher inoculation doses lead to earlier onset and progression of viral replication. In summary, these results have shown that microgels provide a solid surface that supports the growth and development of neurons and allows infection of HSV-1 single cells in droplets. Using microgels to cultivate single cells was an advanced option for future research in neurobiology and virology[83]. It is important to note that the integration of solar thermal PCR with microfluidic systems has enabled the development of a more complete system for KS diagnosis, as shown in the work of Snodgrass et al. This solar-powered procedure is particularly remarkable because it omits the need for complex laboratory equipment and thus improves accessibility and user-friendliness (see Figure 2). The portable KS-Detect system uses solar heat in the denaturation, expansion, and annealing stages of PCR to amplify and detect nucleic acids associated with Kaposi sarcoma. Its microfluidic platform contained various components, including a peristaltic micropump, a focus lens with an alignment device, reagents and a microcontroller. The system's accuracy and sensitivity were measured by gel electrophoresis, with results of 100% accuracy and 90%sensitivity.This technology has the potential to provide rapid and accurate diagnosis of KS for patients far away from medical centers and laboratory equipment [84]. Electrical sensing combined with printed plastic microfluidics can provide a promising approach to detecting herpes viruses while also addressing the challenges in microchip design. By optimizing the design and alignment of the microfluidic system, the sensitivity and specificity of the system can be improved, allowing for more accurate detection of herpes viruses. Additionally, printed plastic microfluidics are a cost-effective and easily accessible alternative to traditional microfluidic fabrication techniques. The integration of electrical sensing with printed plastic microfluidics can provide a low-cost, portable, and rapid detection platform for herpes viruses that can be used in resource-limited settings [85-87]. The microfluidic PCR technique integrates all biological processes, making it easy for users to perform [88]. However, one of the main disadvantages of microfluidic PCR is the inability of these systems to change the number of PCR cycles. Nevertheless, the developed technology has achieved significant success in detecting various viruses such as HIV-1 (subtypes A, B, C, D, E, G, and panel), Kaposi's Sarcoma-associated Herpes Virus (KSHV), and EBV, which is one of the most common infectious viruses. Related studies have shown promising results accuracy and sensitivity, demonstrating the potential of microfluidic PCR in disease diagnosis and

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management. To further investigate, Shafiee et al. a printed flexible plastic microchip was created for the quantitative detection of viruses in plasma and saliva samples. The device can potentially recognize multiple viruses simultaneously on a platform with several channels in line with each other (see Figure 3). Each microchannel has a gold microelectrode that uses a specific antibody for the target virus and identifies it through electrical computation. The study examined parameters such as precision, repeatability, system suitability, performance over time, and robustness [89]. In a recent study conducted by Yamagami and his colleagues, using microfluidic PCR, HSV and varicella-zoster virus (VZV) in tears as symptoms of herpes simplex virus keratitis (HSK) and herpes zoster ophthalmicus (HZO) could be detected quickly. 20 patients were included in this study, 8 patients with HSK and 12 patients with HZO. The test time lasted about 40 minutes and the low detection limit for both viruses indicated the beneficial effect of microfluidic PCR [90]. The created of the Vacrel®8100 chip by Horak et al. in 2014 marked the first time a remarkable immune chip was designed specifically for detecting EBV using an electrochemical method. This chip was built with a flexible, photoresist, dry film material that could be processed under non-cleanroom conditions, as well as an advantage in terms of ease of use and accessibility. The chip could determine the outcome using just 5 µl of human serum sample within 2 minutes, and its response percentage in the same state was found to be 97.5% when compared to the results of ELISA. These results suggest the potential of immune chips in the rapid and accurate detection of viral infections [91]. Biosensors have obtained notable attention due to their ability to detect viruses and other biomolecules with high sensitivity and specificity. They have been utilized in various fields, like medicine, environmental monitoring, and the food industry for, rapid and precise detection of pathogens and other target molecules [92]. The integration of biosensors with microfluidics has become increasingly attractive due to their complementary features and potential for seamless combination. One example is the work by H.H. Kim et al., who expanded a micro-cantilever biosensor to detect HPV infection early. This approach integrated a piezoresistive sensor in the surface of the micro-cantilever, which was modified with gold. Magnetic beads were presented to the sensing zone with DNA fragments of the HPV, and using external magnetic fields enhanced detection sensitivity. The resulting detection signal was amplified and measured between 1.78-1.93 mV [93]. Many animals are susceptible to herpesvirus infections, including cattle, dogs, pigs, horses, and sheep. Some examples of animal herpesviruses include bovine herpesvirus 1, pseudorabies virus in pigs, equine herpesviruses, and ovine herpesvirus 2. These viruses can cause a range of diseases in their respective hosts, including respiratory disease, abortion, encephalitis, and skin lesions, among others [21]. Sexually transmitted infections, such as bovine herpesvirus 1 and equine herpesvirus 3, can be difficult to diagnose in animals, and their transmission can be difficult to control. In addition, these infections can cause remarkable economic losses in the livestock industry [94, 95]. For the first time, Yang et al. demonstrated the use of a paper origami microfluidic platform for the diagnosis of Bovine Herpesvirus-1 (BoHV-1) infection from semen samples. This point-of-care sensing device showed incredible levels of sensitivity and specificity in diagnosing BoHV-1, making it easy to use and well-suited for on-site testing (see Figure 4)[96]. Creating low-cost and precise diagnostic systems for animal diseases is important for effective disease management and prevention. Microfluidic and biosensor technologies have the possibility to supply fast and trusty diagnoses of various animal diseases, which can help to hinder the spread of infectious diseases and improve animal health. In addition, these technologies may also aid to reduce the costs associated with common laboratory testing methods [97, 98]. However, the use of paper-based microfluidic systems is not without obstacles. The main drawback of paper-based microfluidic systems is that patterning channels on the platform can be a challenging and long time. This is for the reason that the paper substrate is not as robust as other materials used in microfluidic devices, like glass or silicon. Consequently, it cannot be easy to reach the necessary precision and stability in channel patterning. This can lead to variability in experimental results and extend the complexity of the microfluidic networks that can be fabricated on paper substrates. One of the most promising advantages of using microfluidic platforms is the ability to utilize the properties of gas and liquid samples at a microscale. By using small volumes of reagents and samples, microfluidic platforms can reduce the overall cost of the analysis and minimize waste generation and samples, significantly reducing the global cost of usage [88]. But these magical small systems have challenges and problems along with many advantages. One challenge in microfluidic systems is the making process, which requires high precision and precision in creating microstructures, often using expensive equipment and materials. The design and optimization of microfluidic devices can also be complex, requiring extensive computational modeling and experimental validation [99]. The next complication that can occur when working with microfluidic systems is the possibility of material leakage, which is more likely due to these systems' small size and large surface area to volume ratio. There can also be pressure differences in different parts of the system, which makes things even more complex [100].

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Figure 1

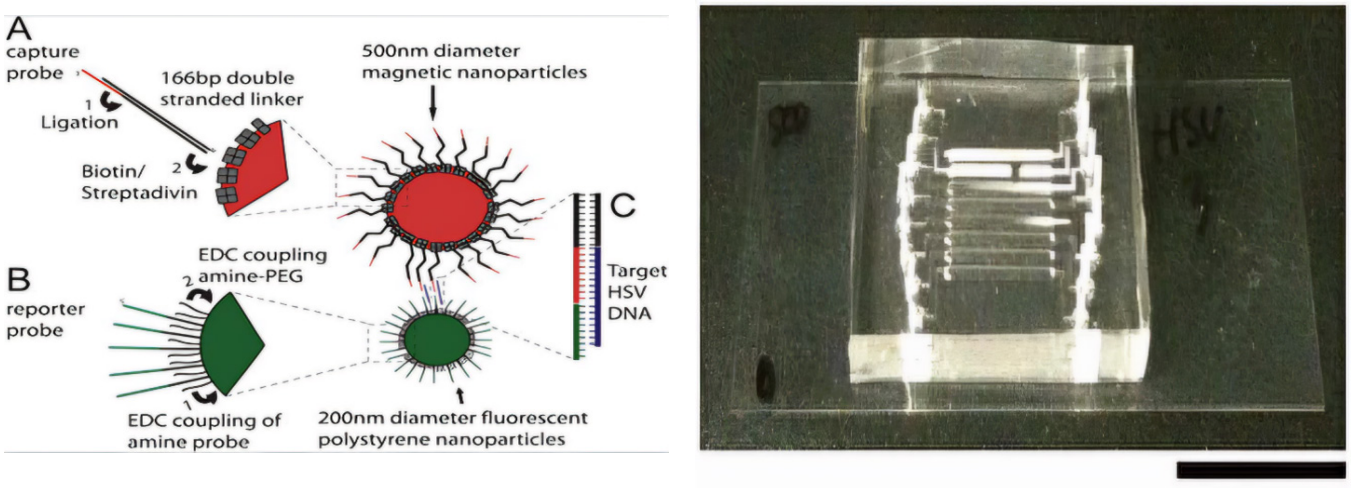


Figure 1: (a) Schematics of nanoparticle functionalization with HSV-specific capture and reporter probes; (b) microfluidic device design, developed in polydimethylsiloxane. Reproduced with permission from[81].

Figure 2

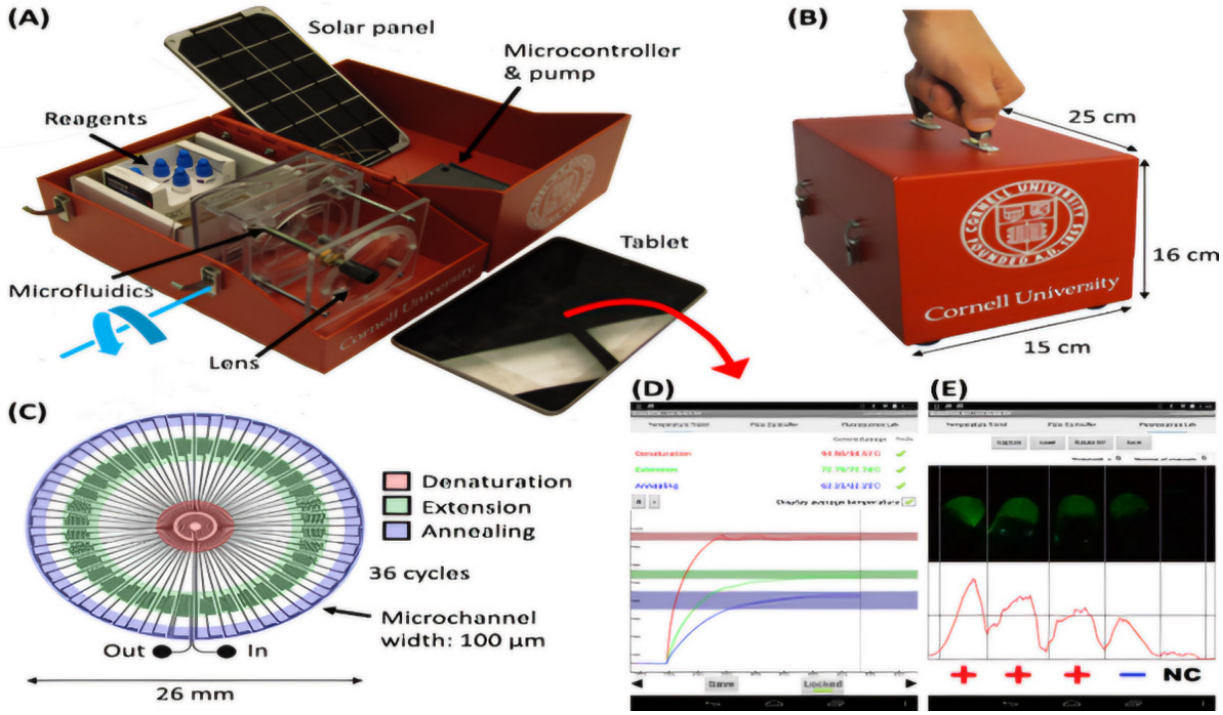


Figure 2: The KS-Detect system. (A) The system embraces all components necessary for solar thermal PCR and subsequent analysis, including solar panels, tablets, and reagents. (B) The system affords easy transportation to patients far away from Health centers. (C) According to the design of this part, Samples are cycled between the higher temperature center of a PDMS chip and the lower temperature edges. (D) A specific Android application is applied to track each temperature zone within the microfluidics and to (E) examine outcomes via fluorescence levels imaged by a smartphone or tablet. Reproduced with permission from [84].

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Figure 3

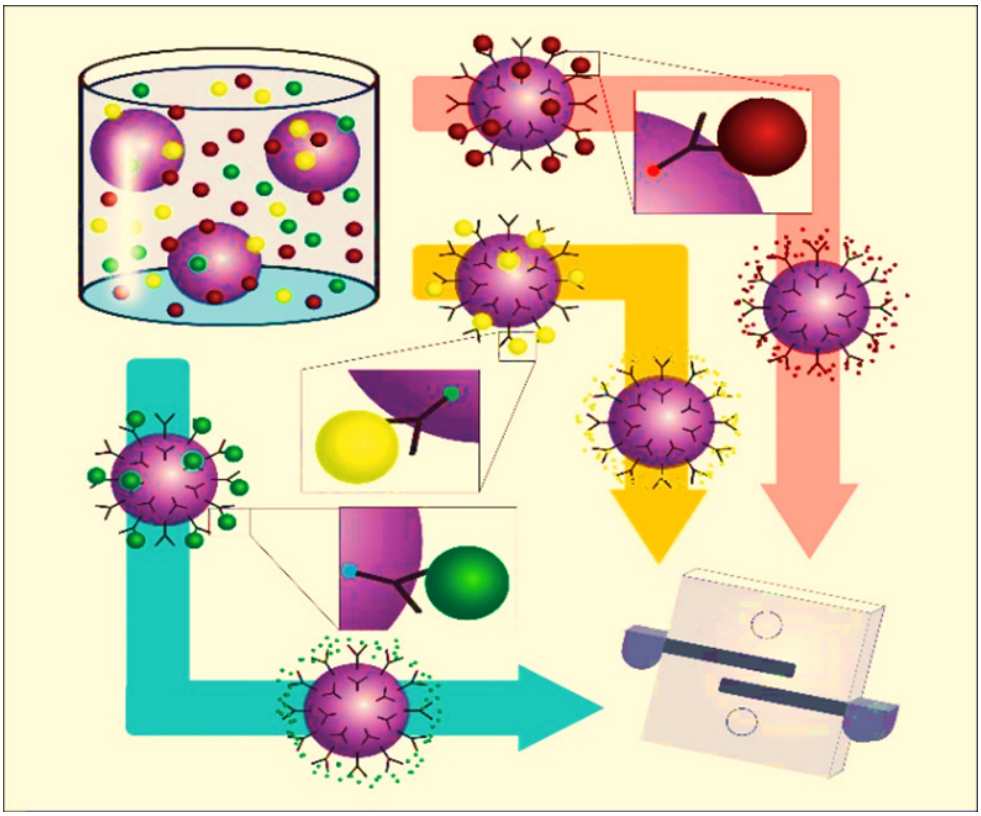


Figure 3: 3D schematic of multiple viruses simultaneously captured and detected by microfluidic device

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Figure 4

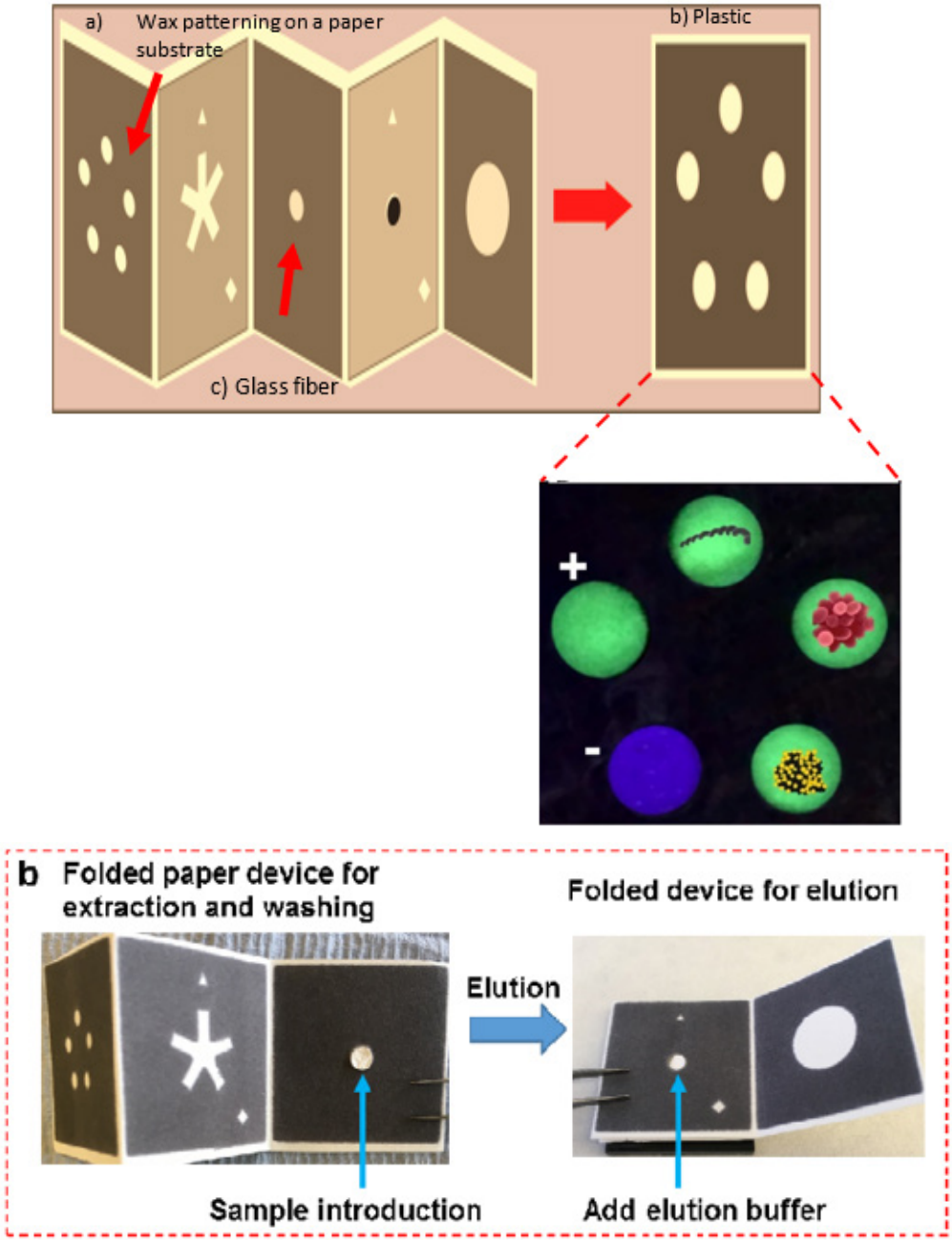


Figure 4: I) Paper origami device for detection of BoHV-1, fabricated from three parts: a) Wax patterning on a paper substrate, b) plastic microfluidic device with LAMP reaction, c) glass fiber membrane. II) b) DNA was extracted by inserting the sample into glass fibers and finally, after performing the absorption LAMP reaction, it was read at 365 nm.

5. CONCLUSION AND FUTURE PERSPECTIVE

The growth of microfluidic technology has displayed notable potential in the field of viral disease detection, including herpes viruses that using microfluidic systems revolutionized the traditional methods of diagnosis by providing a more rapid and effective way of testing. These technologies have also enabled the development of portable diagnostic devices that can be used in critical situations, like prevalence and pandemics, where rapid diagnosis and response are crucial. Although microfluidic systems have shown major capability in various applications, they have not yet been fully integrated into human and veterinary medical diagnostic systems. Even so, they are considered an attractive option for use in acute situations that require non-invasive access, such as point-of-care testing or far-away monitoring. The ability to perform rapid and accurate diagnosis with minimal

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sample preparation and handling can improve the course of healthcare and disease management. Despite significant progress in the field of microfluidics, there are still challenges to overcome. One of the biggest challenges is the cost of developing microfluidic devices for different virus diseases. Creating fully automated platforms that can simultaneously analyze multiple pathogens and biomarkers is crucial. This development would not only reduce the cost of testing but also increase the accuracy of diagnosis. The integration of microfluidics with other devices, such as nanomaterials, PCR, and smartphones, has shown good results in enhancing the specificity and sensitivity of detection. In addition, the merger of microfluidics and high-throughput technologies can lead to the development of faster and more impressive diagnostic platform.

Author Contributions

All three authors have contributed equally to this paper.

Conflicts of interest

The authors declare no conflict of portion

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