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Analytical methods for detection of human cytomegalovirus clinched biosensor a cutting-edge diagnostic tool

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ABSTRACT

Human cytomegalovirus (HCMV) is a beta herpes-virus, which affects human being as a lifelong infection. HCMV is the prominent cause for the infections of congenital with a 1.0–2.4% incidence of live-births, along with possible severe classic cytomegalovirus. Crucial HCMV infection is usually asymptomatic in healthy hosts but it can cause severe or sometimes fatal illness in immuno-compromised neonates and individuals. Various conventional methods such as PCR, virus isolation, antigenemia test, histological and serological are available for detection of HCMV. Among all the analytical techniques, biosensors clinched as the most advanced technology, which offers many features such as simplicity, inexpensive, highly sensitive, and effective approach. The future of diagnosis will rely on the development of point-of-care devices, which can be used at the site of need, resource-restricted settings, and provides affordability. This review describes various analytical methods for the detection of HCMV emphasizing biosensing methods.

1. Introduction

Human cytomegalovirus (HCMV), also referred to as HHV-5 in some literature is a ubiquitous beta - herpesvirus. The virus undergoes periods of activity and latency in the body of the host indeterminately and can be reactivated due to many stress factors [1]. HCMV consists of a dsDNA genome that is wrapped with an iscodelta-hedral shaped protein complex, known as a capsid [2]. The DNA together with capsid forms the nucleocapsid, which is then covered with a protein layer known as tegument. A lipid bilayer (envelope) further covers the tegument nucleocapsid (Fig. 1). Likewise, the glycol-proteins are also embedded within the envelope, which allows the virus to interact within the host cell [2].

Among all the herpesviruses, HCMV is relatively sensitive towards low-pH, agents which are lipid-dissolving and heat. The half-life of human cytomegalovirus at 37 °C is 60 min, which is comparatively unstable at 20 °C. To maintain its infectivity, it requires being stored at -70 °C. HCMV induces infections extending from mild subclinical infections to extreme congenital irregularities and interstitial pneumonia in immune-suppressed patients. A crucial infection of HCMV is mainly asymptomatic in healthy hosts which can cause severe disease and eventually fatal illness in immune-compromised neonates and individuals [1]. HCMV is the prominent cause for the infections of congenital with 1–2.4% incidence of live-births, along with possible severe classic cytomegalovirus.

The brain damage, loss of hearing in children, and significant health issues of HIV-infected patients and transplant recipients are the infectious causes of congenital CMV [2]. Intrauterine infections of HCMV can cause microcephaly, hepatosplenomegaly, and significant morbidity, involving low birth weight, encephalitis, chorioretinitis and mental retardation [2]. In immune-competent adults, the virus remains hidden in a wide range of cell types including lymphocytes and myeloid lineage cells, as well as smooth muscle cells and endothelial cells, which lines blood vessels [3, 4]. In these individuals, early treatment with antivirals can control the infections of HCMV but some difficulties occur like-toxicity, the emergence of anti-viral drugs resistant strains [3]. HCMV is connected through cancers like colorectal, breast, and brain [4].

HCMV is the most prevalent among some groups of population such as old people, immune-compromised and younger people [5]. However, it was found that HCMV prevalence increases with the age. Though prevalence in developing countries also dependent on the socio-economic status and sex preferences among individuals [6]. As in a particular study, the infection in the population was found to be approximately 60% in developed countries while it was 100% in developing countries [7, 8]. There can be a chance of transmission from mother to

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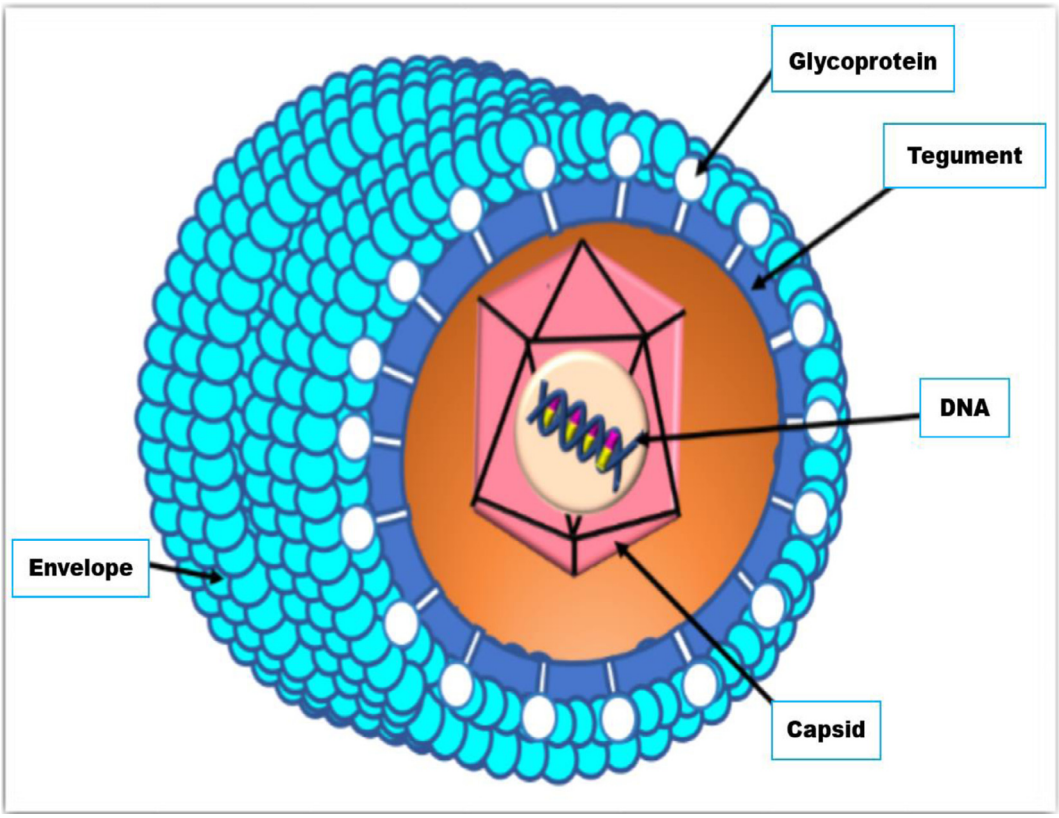

Fig. 1. Structure of human cytomegalovirus representing its components.

neonatal through transplacental, secretion from cervical or vagina, and through mother's feed after birth. However, the aforementioned transmission also depends on socio-economic status [5].

The molecular techniques are the most prominent method used for the quantitative diagnosis of viruses, mainly in the specimen's peripheral blood [9]. Among current clinical methods, RT-PCR is the best viable and accurate technique for the diagnosis of viruses. The RT-PCR shows better results, easy to use, and has a somewhat improved ability to quantify the targets. Moreover, PCR coupled techniques also came into existence for the detection of viruses [10-14]**.** However, point-of-care devices are still required to fulfill the demand for early and fast diagnosis. Various biosensors have been developed, which overcome the limitation associated with the conventional methods [15-24]. The future of diagnosis will rely on the development of point-of-care devices, which can be used at the site of need, resource-restricted settings, and provides affordability. The answer to these entire requirements is biosensors, as they offer all the aforementioned characteristic features.

In this study, we have presented the existing human cytomegalovirus-RNA, virus particle, Ag and Ab diagnostic methods. The issues around false-positive and false-negative outcomes in clinical practice, as well as unsolved were also addressed. Furthermore, new detection methods were discussed, such as novel nanoparticle-based different biosensors that may be used in the future to increase the efficiency of human cytomegalovirus detection assays.

2. Various methods of diagnosis of HCMV

2.1. Molecular methods for detection of HCMV

There are various diagnostic methods available for the detection of HCMV including molecular methods such as PCR [10], virus isolation [25], antigenemia tests, and various other serological tests [26].

Among all methods, PCR is the most exploited method for the detection of various viral diseases [27]. PCR for the detection of CMV DNA has the potential aptitude to detect a low variable sequence of DNA. Moreover, the method can be used for the detection of HCMV DNA in various samples such as plasma, whole blood, Broncho-alveolar lavage fluid or cerebrospinal fluid [27]. Real-time PCR was employed for the detection of Cytomegalovirus infection by Ross et al., using saliva and urine samples from a large cohort of infants, which gave 98% positive results [28]. The serological tests provide detection of CMV IgG or IgM, which can identify the patient, had a previous infection or not. Numerous serological tests are combined with direct hemagglutination, ELISA (enzyme-linked immunosorbent assay), radioimmunoassay, anti-complement immunofluorescence and complement fixation [25]. David et al. described the serological detection of HCMV - DNA in the serum of human tonsillar lymphocytes using three approaches in efforts to validate presence of HCMV in tonsils: (i) Isolation of virus from cultures of the cell, (ii) Immuno-histochemical staining immune- peroxidase technique intended for the detection of viral antigens (iii) DNA dot- hybridization through HCMV- DNA probe intended to identify viral- DNA. They concluded that infectious HCMV and other viruses were not isolated in cell–cultures and no viral antigens were sensed by immune peroxidase staining in the tonsillar tissue [29]. The serological tests are not that sensitive, as they have a false result probability of about 5% [25]. Virus isolation is one of the oldest methods used for the detection of HCMV. Amini et.al. were isolated HCMV from a new human fetal foreskin fibroblast-derived cell line. The cytomegalovirus developed mainly in diploids fibroblasts cells of humans, which cultured at 36 ˚C for 1–3 weeks and analyzed to identify HCMV inclusions in fibroblasts. This method required 15 days to show the results, thus making it extremely unsuitable [30] (Table 1).

The quick and initial detection of pathogens is the key to set the best anti-infectious therapy. However, even problematic is strain variation and the increasing degree of multiple drug resistance, which are

Table 1
Summary of procedure, advantages and disadvantages of conventional methods of HCMV.

	Polymerase chain reaction (PCR)	Virus isolation	Antigenemia test	Histological analysis	Serological test
Procedure	Detection of nucleic acid target sequence [32]	Isolation of the CMV virus from a clinical sample [25]	The quantitative analysis gives the idea of a viral load that helps in monitoring patients before and after therapy [33]	Characterize intranuclear inclusions in CMV [34]	Identification of CMV IgG and IgM [25]
Advantages	Efficient in the detection of CMV DNA in various samples like-plasma, blood, Broncho-alveolar lavage fluid or cerebrospinal fluid [27]	Sensitive for CMV DNA in various samples [35]	Useful for assessing the possibility of ailment development [36]	The method is very simple and non-expensive [37]	It is very rapid so that a result can be available within the same day
Disadvantages	Increased analytical sensitivity leads to lower clinical specificity [38]	Requires total asepsis [39]	It is labor-intensive and does not approve of automation [40]	This method does not apply to all specimens. Test errors and false-negative results are vulnerable [41]	Gives reasonably accurate results within a minutes [34]

hitting forward a great challenge to clinical works. Due to the variation in strains and drug resistance become more inescapable, the prevention and control of viruses have been a thoughtful issue in recent years [31]. Therefore, there is a need for a point of care (POC) analysis that is much more rapid and precise. POC devices also reduce the burden on hospital staff during epidemics. In present era, various sophisticated POC came into existence but yet to be commercialized. Therefore, if efforts are made in this direction then it can be a boon to the global population. The diseases can be effectively managed only when it is diagnosed at an early stage. This is why biosensors appear as an excellent alternative or as a complementary analytical tool of diagnosis. Table 1 summarizes the procedure, advantages and disadvantages of various conventional methods for detection of HCMV.

We emphasize herein the usage of biosensors for the detection of cytomegalovirus.

2.2. Biosensing methods/Biosensors for detection of HCMV

2.2.1. Concept of biosensors

The biosensor is a device, which comprises a biological recognition element, engaged in the direct-spatial interaction through IUPAC definition i.e., transduction system. Biosensors can operate as devices that change a biological and physical event into a measurable signal [42]. It involved a bio-sensing component like- living cells, enzyme tissue that offers selectivity and a transducer, which changes the chemical responses into a processable signal [43]. In detail, the biosensor involves three elements- the first element is the bio-mediator i.e., a biologically derived material such as biological sensitive elements, cell receptors, nucleic acids, organelles, microorganisms' tissue and antibodies [44]. the second element is transducer i.e., piezoelectric, electrochemical and optical, which converts the signal by the help of signal resultant from the interactions of analytes through the signal from biological-element, which is measurable. The third element is a signal processor / allied electronics, responsible for easily monitoring the result of the visualization method [44]. Certain biosensors need a procedure of bio-mediator immobilization to the surface of the sensor (glass, polymer, metal, polymer and other materials) with the help of chemical/physical methods [45] as shown in Fig. 2.

Different biosensors for the detection of HCMV were compared according to the limit of detection (LOD), linearity, sensitivity, and specificity. Each parameter influences the functionality of the device. However, among every measurement, the LOD and linearity play a significant role in the fabrication of the device. Biosensors should be able to detect a very low amount of the analyte and clinical values [46]. The LOD shall be represented in units of concentration and shall signify the minimum quantity of analyte in the sample, the concentration of which may be calculated with appropriate precision and accuracy within certain experimental conditions. The LOD is widely used as proof of the consistency of the biosensor, i.e. the lower the detection limit, the greater the applicability of the device [46]. Other parameters such as specificity and precision also depict the functionality of the biosensor. The biosensor should be highly specific towards the specific analyte so that false negative and false positive results could be avoided. Fig. 2 represents the various components of biosensors indicating bio-recognition elements, types of transducer and amplifier.

2.2.2. Types of biosensors for optimum detection of HCMV -DNA

Electrochemical DNA biosensors for optimum detection of HCMV -DNA: DNA is suitable for the application of biosensing, as the base-pairing interactions among complementary sequences are robust and accurate. In a standard setup, immobilization of ssDNA probe sequence inside the detection layer, at which place the base-pairing interactions required DNA target to the surface [47]. The repeated, effectively uniform structure of DNA makes its assemblage well-defined upon the surface of identification. At this interface that critical dynamics of target and capture take place to generate the detection signal, thus, predictable immobilization of probe sequences of nucleic acid, while preserving their latent affinity to DNA target is essential to the whole performance of the devices [48]. However, this recognition occurrence is recorded at last that depends on the transduction of the signal process, whether it is mechanical, electrochemical, or optical [48]. With the aforementioned principle, biosensing methods such as microarrays, DNA chips and DNA sensors tend to attain interest, as they can detect target DNA with higher sensitivity [48]. An electrochemical identification of the DNA system has been produced to quantify the amplified 406-base pair DNA sequence of HCMV sensitively. This technique was focused on the target, HCMV DNA hybridization with Au-NPs modified oligonucleotide, accompanied by the liberation of Au by acid therapy. Therefore, the evaluation of the solubilized AuIII ions via ASV with a sandwich-type Screen-printed microband electrode (SPBME) through CV indirectly was performed. Its conjunction of the sensitive Au-III evaluation at SPMBE with the high amount of Au-III released to every AuNPs probe, enables the identification of 5 pM of amplified HCMV DNA fragment [49]. But somehow additional developments are needed likewise for the production of the compacted, simple to use, easily handled device, or with the target of working in the lesser assay volumes and also takes lesser response time for increasing the sensitivity and low limit of detection. To overcome this drawback, Narang et al. proposed a DNA sensor employing an amperometric microfluidic paper-based analytic device (EPAD) combined from zinc – silver nanoblooms and the probe HHV- 5 DNA for the diagnosis of human herpes originated via cytomegalovirus also called human herpesvirus (HHV-5) [50]. Through this research analyte transfer from the volume of sample to the element for bio-recognition, reduced proportions and volumes were shown to be improved. Distances among bio-recognition elements and analyte were also reduced in this approach, which leads to a decrease in response time. EPADs have shown to be highly selective and

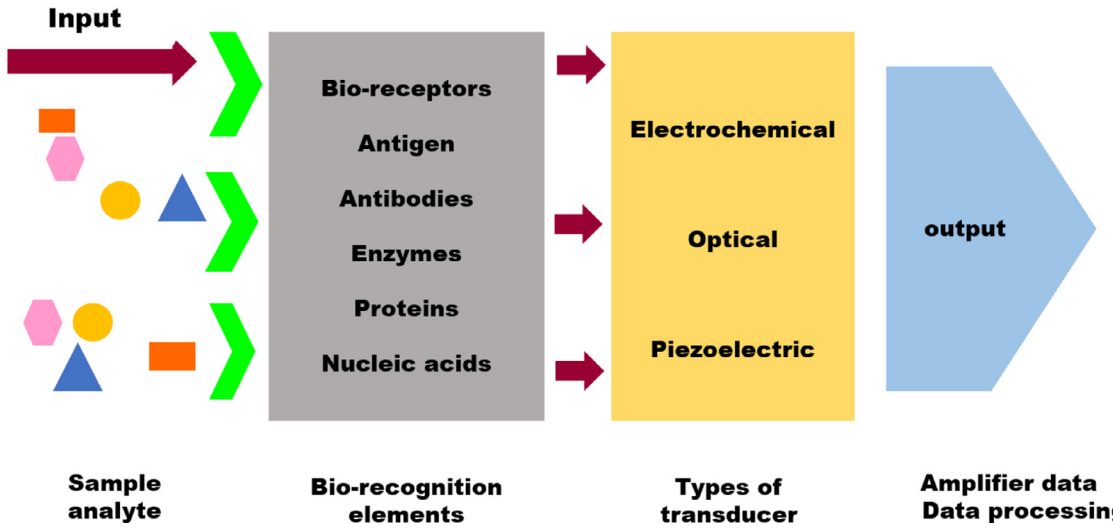

Fig. 2. Flow chart of representation of components of biosensors indicating bio-recognition elements, types of transducer and amplifier.

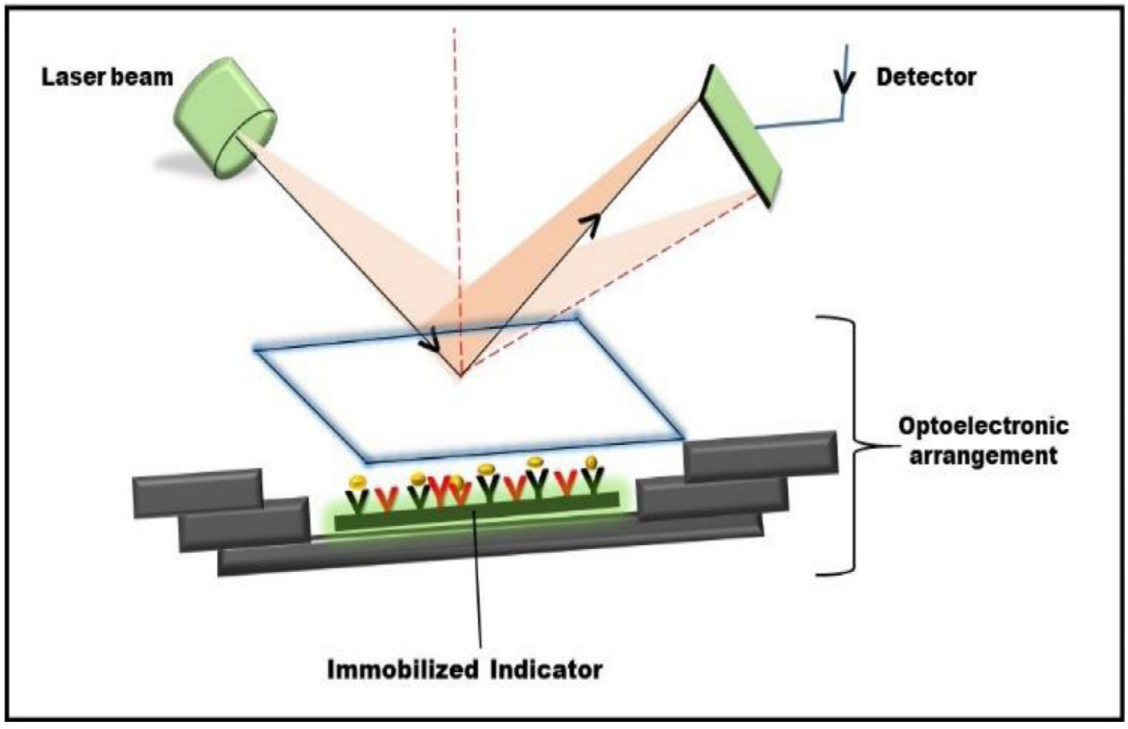

Fig. 3. Diagrammatic representations of components of optical biosensor.

have marketing potential, as it is simple and has amplified sensing and reducing interference from electro-active species. There was a limit of detection i.e., 97 copies per ml and holds a great value to clinical evaluation [50].

Optical sensing of HCMV through surface plasmon resonance method: In optical biosensors, the detection of analytes is facilitated through optical fibers based on absorption, scattering of light, or fluorescence. These biosensors are most attractive, as they are accomplished by doing the multiplexed diagnosis [50]. In these optical biosensors, waveguide devices and optical fibers are used to improve detection sensitivity by increasing the interaction between the sensor surface and the guiding light (Fig. 3). The different types of analytes can be identified utilizing various record wavelengths. These biosensors have found applications in various in-vitro phenomena. An optical biosensor essentially identifies and quantifies the changes in various characteristics of a sample such as fluorescence, phase shift, absorbance, and reflectance. By simply using fluorescent dyes or labeling of aptamers with fluorescence, optical detection can be performed [51]. Gietmann et al. showed the interfacing between the peptide and immobilized protease of HCMV, which was characterized through biphasic surfaces of surface plasmon resonance (SPR) signal-dependent bio-sensor. In this method, an enzyme is immobilized on the surface of the sensor chip by coupling of amines resultant in the active enzymes through high catalytic-efficiency as compared to the enzymes present in the solution, because of the lower k_m value. The

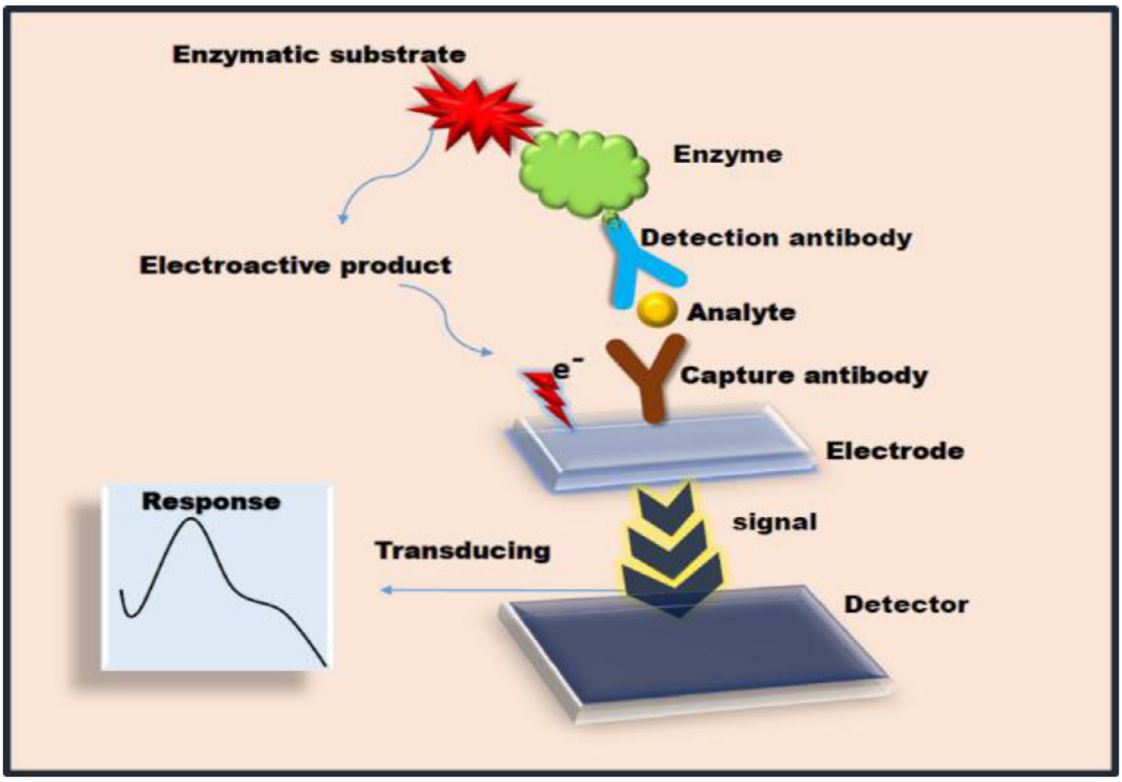

Fig. 4. Schematic representation of a sandwich immunoassay.

monitoring of small changes in a chip surface mass, this direct real-time binding test offers quantitative and qualitative data on biomolecular interaction. Observing the immobilized enzyme's hydrolytic activity, they assessed its overall significance for interaction analysis. The simultaneous binding detection and conformation changes using optical biosensor technology are expected and conformational changes are of great importance for the added characterization of the enzymatic properties of HCMV protease and to the diagnosis of inhibitors of specifically this enzyme [52].

Piezoelectric affinity sensor for detection of HCMV underlining strand displacement amplification method: A Piezoelectric affinity sensor was established by Susmel et al. to detect the IgB epitope of HCMV [53]. The sensor employed antibodies immobilized onto the Au electrode. The developed biosensor was disposable and the sensing mechanism of detection was immune fluorescence on a functionalized surface. The drawback of this method is a low sensitivity, which restricted its applicability to samples with low viral loads. The benefits of this technique are that it is inexpensive, fast and highly specific, because of Ab/Ag interaction [54].

Chen et al. introduced a strand displacement amplification (SDA). It is a well- designed nucleic-acid amplification method, which could operate under the terms of a constant temperature. They developed a robust liquid phase identification system, in which the crystal oscillator plate was mounted using a simply adjustable screw-a-threaded clamping method and positively implemented the new sensor system to the real-time HCMV SDA monitoring system [55].

Immunosensor for detection of HCMV through electrochemical and optical transduction: Immuno-sensors are appealing diagnostic tools which are being widely used in biological research due to their high-affinity interactions between antigens and antibodies [55]. The electrochemical immunosensors combine the specificity of antibody-antigen detection processes with exemplary electrochemical transduction capabilities linked to high specificity, speed of analysis, and compatibility with mobile devices (Fig. 4). By relating the benefits of biosensor area along with the difficulties HCMV detection, mainly in human samples, like- easy and disposable electrochemical immunosensor was developed to detect the glycoprotein-B of HCMV in the urine sample. The glycoprotein-B (gB) was chosen as an antigen of electrochemical immunosensor. Since gB is prevailing Ag into the human cytomegalovirus envelope, about 100% of infected patients produce Abs against gB protein. HCMV gB may be considered as a prominent factor for utilizing for the production of HCMV detection assay. Using above mentioned approach, a method was proposed, which was dependent on the immunoassay i.e., sandwich – based type with secondary antibody (Abs) labeled with Au NPs, that permits the AgNPs precipitation leading to enhanced immunosensor's sensitiveness. Glycoprotein-B detected via electro-chemical stripping-analysis of AgNPs quantitatively, set down upon immuno-sensor by catalysis through nano-gold labels [56] The numerous parameters like-BSA concentration, antibody concentration, silver enhancer concentration, the silver deposition time, and glycoprotein B incubation time were adjusted. This sensor showed a detection limit of 3.2±0.2 ng/mL. The sensor was employed in real samples like - urine in which the biological matrix does not inhibit through immune-sensor diagnosis capability [56]. But, the reproducibility of such an approach wasn't very effective due to the randomized immobilization of the primary antibody on the working electrode that culminated in limited antigen recognition efficiency, weak signals compared to the large proportion of this antibody utilized.

To rectify this problem Pires and coworkers employed glycoprotein – B to develop a simple enzyme immunoassay based on magnetic- particle (mpEIA) for the identification of the glycoprotein B of CMV in urine-samples. Magnetic- beads were altered with the protein - G (MBs-PrG) and were employed in immunoassays for promoting the immobilization of antibodies on the solid- phase. The sensor arrangement was based upon the analyte i.e. B-HCMV sandwiched between the primary MAb (MBs-PrG-mAb1) and secondary anti-gB-HCMV antibody (Ab2-HRP) to

permit the spectrophotometric detection. The estimated detection limit for gB-HCMV was 30pg / mL, which appeared to be very favorable for further assessment. The time required for the detection was about 3 h making it a more rapid and simple method of detection [57]. The detection of glycoprotein-B by the electrochemical-immunosensor in urine for detecting HCMV is a modest and disposable technique for human samples [58].

CMV- pp65 antigen also occurs during the period of early stages of the disease or is scattered into the human vascular endothelial cells (HVECs), peripheral blood leukocytes (PBLCs), or body fluids through quick, effective and extremely specific method for early detection of CMV pp 65. Huang et al., 2016 reported a specific electrochemical amplification immune-sensor for the identification of Cytomegalovirus - pp65 antigen created upon Pt and Pd nanoparticles functionalized single-walled carbon nanotubes nano horns (Pt-PdNPs@SWCNHs). SWNCHs are new- a type of carbon nanomaterial showing benefits like lower impurity levels, fewer adverse effects and higher surface area. However, SWNHs have a much smaller pore size that could easily absorb small molecules and ultimately improve catalyst stability. The fabricated sensor was able to identify different concentrations of CMV pp65 antigen under optimized environments showing linearity of 0.1–80 ng/mL and a low detection limit i.e., 30 pg/mL and displayed brilliant selectivity as well as stability. Therefore, this sensor could be employed as a valuable method for the early and point-of-care detection of HCMV infection in clinical trials [59]. Another biosensor for the detection of CMV pp65 was reported by Lei et al., in 2018 based on SnS_2 quantum dots (SnS_2 QDs), which were new emitters for the ultrasensitive detection of the CMV- pp65 antibody (anti-CMV pp65) via smart circular peptide-DNA nano-machine amplification. Compared to the above-stated immunosensors, this sensor's detection limit (LOD) was lower (0.33 fM) with higher sensitivity and specificity [60].

Further, for the quantitative and high throughput multiplexed analysis, BIE (biosensor based on imaging ellipsometry) could consider being evident. Sun and coworkers in 2015 reported an immunosensor for the identification of CMV antibodies in human serum based on BIE. CMV antigen i.e., CMV-3A was cast on silicon and employed to capture antibodies against CMV in the serum. An antibody used against the antigen was human immunoglobulin G (anti-IgG). The sensor displayed LOD of around 0.01 IU / mL, which was an outstanding approach to the point of care research [61].

A label-free immunosensors have nowadays attracted much interest. Graphene nanosheets, which are 2D honeycomb lattices of sp_2 – bonded carbon atoms have attracted tremendous attention recently, because of their unique thermal, mechanical and electrical properties. It was reported by Zeng et al. in 2016 for the determination of PP65 phosphoprotein antigen. Screen-printed carbon electrode was altered with a nanocomposite made from multiwalled carbon- nanotubes (MWCNTs), graphene nanosheets, and chitosan and 1-butyl-3-methylimidazolium hexafluorophosphate [62]. AuNPs were immobilized on the electrode and incorporated into layers of the polymeric redox mediator thionine. The monoclonal antibodies raised against PP65 were immobilized on the surface with amine-gold interaction. Then finally, horseradish peroxide (HRP)was employed for the blockage of the remaining active sites on the surface of AuNPs and to work as an enzyme in immunoassay. The monitoring of electrocatalytic reduction of hydrogen peroxide by HRP was done by pulse voltammetry. Under improved conditions, the differential pulse voltammetry signal on typical working voltage of 0.5V, decreased with increasing concentration of PP65 from 0.12 to 300 pg /mL and displayed a LOD of 30 pg/mL. Thus, the stated electrochemical immunosensor could be a potential approach for PP65 detection [62].

Relatively, the danger of postnatal infection may be significant for premature offspring in particular by breast milk. To resolve the issue of breastfeeding adaptation and in the existing context, Py et al. proposed an HCMV biosensor, based on sandwich ELISA principle in a dynamic flow configuration (lateral flow immuno chromatography). The paper discusses research that has just begun for the detection of HCMV in breast milk, which is the potential to set up a simple in utilization and fast point of care (POC) tool. This would allow for a corresponding adaption of the strategy for feeding milk, as the peak of HCMV infection arises after 4 to 8 weeks of birth. The detection of HCMV utilizes a particular secondary Abs coupled from HRP that ultimately identifies the virus being captured. After adding the substrate of an enzyme, a colorimetric reaction occurred which allowed the conversion of substrate to a blue-colored product. The LOD was 2.8×10^{-4} μA and holds the potential for further studies for the detection of HCMV by their cost-effective, easy – manufacturing, and lightweight portability for in-field measurements [63]. Pires et al., 2019 developed an easy, responsive, reversible, and compact system based on the analyte protein gB sandwiched between the primary monoclonal antibody and the secondary anti-gB-HCMV HRP labeled antibody, thus retaining the immunoassay scheme. The researchers used magnetic particles that had been functionalized with protein G (MBs-prG). The immunosensor developed was shown to be a compact, fast, precise, reliable, low-cost, and efficient method of detecting gB in human urine samples for the useful diagnosis/screening of HCMV infections [64].

The biosensors based entirely on paper are becoming increasingly popular. It is, however hard to keep NPs in the paper substrates devoid of binding them irreversibly from the cellulose matrix. This made it interesting to create biosensors in paper-based reservoirs that integrate nanoparticle probes. Alba – Patiño et al. attempted to solve this restriction using a novel technique for depositing protein decorated NPs on the paper – substrates, which also permits to release them on demand. This includes spotting NPs onto pieces of filter paper modified with polystyrene sulfonate. The avidin-modified gold NPs and Abs can be simply shifted to a receiving wet piece of paper from a dry reservoir by easily pressing with a finger or clamp. The paper-based immunosensors integrating the reservoir, allowed the identification of gB from HCMV in serum with a LOD of 0.03 ng/ mL and a total assay time of 12 min. The LOD attained with a short test time, together with the reservoir's longer shelf life, made the suggested paper – only biosensors ideal for point – of – care analysis [65].

3. Conclusion and future perspectives

CMV-related disease follows a persistent development in the lack of active theragnostic involvement. Consequently, there is a general requirement for sensitive, selective and rapid detection of active CMV infection. The present review emphasizes the impression of biosensors and their sub-class of biosensors to detect HCMV. With perspective knowledge of diagnosis of the HCMV, it can be concluded that the electrochemical methods are the finest alternative for all the necessities. Electrochemical methods provide selective, sensitive and fast diagnosis of HCMV. Moreover, numerous miniaturized devices are also exploited for electrochemical sensing. Currently, paper-based biosensor has transformed the sensing, as it provides many beneficial features like- cost-effective, fast response, facile approach, non- tedious and minimal requirement of the sample. The electrochemistry has advantageous characteristics over the other current measuring systems, because on-field detection of electrochemical biosensors can be fast, simple, and low cost. Its distinctive characteristics show various novel functionalized electrochemical biosensors. During the past few years, there is an definite improvement of sensors. Furthermore, in the current scenario, a very new method came into light during the global pandemics of COVID-19, which can be applied to the diagnosis of various viral diseases. The CRISPR (Clustered regularly interspaced short palindromic repeats)-Cas system, a new diagnostic tool which is sensitive and specific. This model has been effectively exploited for the diagnosis of COVID-19 and helps in developing POC devices. Therefore, the above method can also be applied for the diagnosis of herpes virus and in addition, the device can be interfaced with the smartphone and with telemedicine (Table 2).

Table 2
Summary of different biosensors to detect the HCMV.

Biosensor	Principle	Markers detected	Limit of Detection (LOD)	Advantages	Disadvantages	Ref.
Electrochemical - DNA Sensor	Based on ASV on SPMBE	HCMV DNA	2.225ng/mL	-Fast- Low -cost	-Highly buffered solution may interfere	[49]
	Based on EPAD integrated with Zn – Ag nanoblooms	HHV-5 DNA	97 copies /mL	-The massive device can be produced with a simple and fast fabrication process	-Requires expensive wax printers. -Requires an extra heating step after wax deposition.	[50]
Optical-biosensor	SPR dependent method	Human cytomegalovirus protease and peptide interactions.	-	-Reusable -Label-free -High sensitivity -Simple	-Low selectivity - Non- specific binding to surfaces. -It requires strict observance.	[52]
Piezoelectric Biosensor	Based on the strand displacement amplification technique	The nucleic acid of CMV	-	- Highly sensitive. - Less time-consuming. - Dynamic real-time detection. - Fast	-Its inability to efficiently amplify long target sequences. - Usually for dynamic measurement only. -High-temperature sensitivity	[55]
fImmuno Sensor	Sandwich-immunoassay dependent	gB-human cytomegalovirus in a urine sample and blood samples prepared in buffer	3.3±1.7ng/mL (Blood samples) preparing by using buffer and 3.2±0.2ng/mL (urine sample)	-Acceptable reproducibility	-Low detection range i.e. 5-15ng/mL. - The efficiency of antigen detection is small. - Low signal compared to a large number of antibodies.	[56]
	Based on Sandwich immunoassay	gB-HCMV in urine samples	0.09 ng/ mL	-Rapid -Easily quantified.	-Low stability -Short shelf life -High cost.	[57]
	Based on signal amplification employing Pt and Pd nanoparticles.	Cytomegalovirus pp65 antigen	0.03 ng/mL	-Highly specific. -Highly sensitive. -Highly stable.	-Costly antibodies. -Multiple steps required.	[59]
	Based on electro chemilumines-cence (ECL)	cytomegalovirus pp65 antibody (anti-CMV pp65)	0.33fM	-Sensitive -Modest cost -Selective	-Low optimization time. -Complicated preparation -Low compatibility	[60]
	Based on ellipsometry	CMV-3A	0.024 ng/mL	-Non- destructive measurement - Large measurement range. -Real-time monitoring -Fast measurement - High thickness sensitivity.	-Low spatial resolution. -Difficulty in the characterization of the low absorption coefficient. -Indirect analysis. -Optical model for data analysis.	[61]
	Based on sandwich immunoassay	PP65 phosphoprotein antigen.	30×10^{-5}ng/ mL	-Simple -Cost-effective -Highly sensitive	-Redox species leakage. -Limitation to the indirect detection system	[62]
	Based on sandwich ELISA	HCMV in breastmilk	-	-Simple -Reliable -Highly sensitive	-Expensive cost of instrumentation set-up. - Surface modification is a challenge - Optical devices required. -Low selection range.	[63]
	Based on the analyte protein gB sandwiched between the primary monoclonal antibody and the secondary anti-gB-HCMV HRP labelled antibody	gB-HCMV in urine samples	-	-Simple -Portable -sensitive	-Surface modification requirement	[64]
	Based on nanoparticle reservoir on paper substrates	glycoprotein B from HCMV in serum	0.03ng /mL	- Storage of protein decorated NPs on a paper substrate -Cost-effective	-Low resolution. -Unstable upon heating.	[65]

Declaration of Competing Interest

Authors declare that no conflict of interest.

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