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RECENT ADVANCEMENTS ON EARLY DIAGNOSIS OF CMV INFECTION

GOHIL KJ[1*], JADAV S[1], PATEL D[1], GUJAR K[1] AND UPADHYE VJ[2*]

1: Department of Microbiology (M.Sc.), Parul Institute of Applied Science (PIAS), Parul University, PO Limda Tal.Waghodia, Dist. Vadodara, Gujarat (India)

2: Associate Professor, Department of Microbiology, Parul Institute of Applied Science (PIAS) and Deputy Director, Center of Research for Development (CR4D), Parul University, PO Limda Tal.Waghodia, Dist. Vadodara, Gujarat (India)

***Corresponding Author: Kinnari Jayeshkumar Gohil: E Mail: gohilkinnari2@gmail.com;**

Dr. Vijay Jagdish Upadhye: E Mail: dr.vijaysemilo@gmail.com

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ABSTRACT

The most worldwide cause of non-genetic sensory deafness in children is Cytomegalovirus (CMV), which is also the most mutual cause of congenital infection. CMV spreads horizontally by close contact with biofluids such as urine, breast milk, saliva, and sexual excretions. Pneumonia, nervous system issues such as brain inflammation (encephalitis), and digestive system issues such as colon (colitis), esophageal (esophagitis), and liver (hepatitis) inflammation are all possible side effects of CMV infection. As a result, a CMV infection must be recognized and treated. The early diagnosis of immunosuppressed persons with CMV illness, as well as neonates and babies with congenital CMV infection, stimulated the discovery of innovative diagnostic techniques for the rapid recognition of immunosuppressed individuals with CMV disease. CMV infection is diagnosed using particular antibodies and molecular testing. The Identification of acute maternal CMV infection needs the presence of IgM and a low IgG avidity. CMV PCR of the amniotic fluid is usually used to approve fetal infection. Serological assays are

executed to detect IgG antibodies, with the Enzyme-linked immunosorbent assay (ELISA) being the most widely used technique. The latest available methods of CMV infection are highlighted in this review article.

Keywords: Cytomegalovirus(CMV), Congenital infection, Polymerase chain reaction(PCR), Enzyme-linked immunoassay(ELISA), Antigenemia assay

INTRODUCTION:

Cytomegalovirus is a mutual human DNA virus that belongs to the Herpesviridae family **[1]**. The virus was discovered in 1956 by three different groups of scientists: Rowe and colleagues, Weller and colleagues, and Smith. It is presently well-known as the most prevalent congenital viral infection in humans, affecting 20,000 to 40,000 newborns in the United States each year **[2]**. CMV usually causes an asymptomatic or minimally symptomatic severe disease in immunocompetent patients. Shivering, depression, weakness, muscle soreness, leukopenia, and/or thrombocytopenia in the presence of viremia, or end-organ illnesses such retinitis, pneumonitis, encephalitis, hepatitis, or gastrointestinal tract ulcers can all be manifestations of CMV infection in immunocompromised hosts. These symptoms are linked to a high rate of morbidity or mortality **[3]**. It is the most dominant cause of cCMV infection, in North America and Europe, it affects between 0.5 to 1% of live births and up to 6% of live births in developing countries. Children's non-genetic sensorineural hearing loss (SNHL) is most commonly caused by CMV. The estimated percentage ranges from 5% to 15% in asymptomatic congenital CMV (cCMV) cases to 36% to 90% in symptomatic cCMV survivors **[4]**. CMV is transmitted by body fluids such as saliva, breast milk, urine, and sexual secretions. Among healthy young children in group care or overcrowded housing, chances for person-to-person transmission may be higher, which could rise the prevalence of primary infection. Infected children may continue to shed viruses into their pre-school years, making them a significant source of infection for their adult caregivers (including pregnant women) and other children **[4]**.

How cCMV diagnosis is confirmed?

- 'Gold standard' test: Urine CMV PCR/Shell vial prior to 21 days postnatal stage
- Other positive tests prior to 3 days postnatal age: CMV PCR on

a) Neonatal Dried blood spot (DBS),

b) Saliva PCR, if confirmed through urine or other* **[4]**

DIAGNOSTIC METHODS FOR CMV:

1) Molecular tests:

a) Polymerase Chain Reaction Amplification (PCR):

The polymerase chain reaction (PCR), which is based on nucleic acid amplification, it is a widely available, quick, and sensitive CMV detection method. Although most CMV DNA detection methods focus on the highly conserved regions of critical immediate-early (IE) and late antigen genes, a diversity of other genes have been used as targets. DNA can be removed from whole blood, leucocytes, plasma, or any other tissue (tissue biopsy samples) or fluid (urine, CSF, BAL). The degradation of specimens with time after sample collection is not as important with PCR testing as it is with other CMV tests **[2]**. Depending on how much viral DNA is discovered in the sample, PCR for CMV DNA might be qualitative or quantitative. The qualitative method's threshold must be correctly established to avoid over-detection. Quantitative PCR (Real-Time PCR) allows for continuous monitoring of immunocompromised individuals to identify those who are at risk of CMV infection and assess treatment response **[5-7]**. This process is frequently more expensive than the Antigenemia test, but it is quicker and may be automated. As a result, the number of copies per milliliter of blood or plasma is usually reported. Reverse transcriptase (RT-PCR) may be used to distinguish viral mRNA transcripts in peripheral blood leukocytes in the presence of DNA. Irrespective of whether CMV DNA is present or not, the absence of circulating mRNA is connected to a lack of CMV-related symptoms, whereas its presence is only seen in the situation of sickness. CMV IE mRNA has been recognized in monocytes and polymorphonuclear leukocytes through active CMV infection. However, it looks to be less delicate than the pp65 antigen test and PCR in identifying CMV infection **[2]**. A commercial kit (Bio-Core CMV PCR kit cat. no.: 11091) was used to mark the phosphorylated matrix protein (pp65) gene in the CMV viral genome in the isolated samples. A thermal profile was employed, as specified in the kit description. To ensure the test's validity, all PCR runs included negative and positive controls provided with the kit. To prevent contamination, the Biocore CMV PCR kit contains a dUTP/UDG system. Positive PCR results were seen as a distinct band at 245bp in gel electrophoresis using 1.5 percent agarose with ethidium bromide staining on the BIOMETERA gel

documentation system (Bio-Doc Analyze). Both tests were carried out according to the kit's instructions. The data were statistically examined using Chi-square and Fischer exact tests **[8]**.

NEONATES

PCR Analysis of saliva can also recognize CMV infection. Following positive results, urine PCR testing is used to verify the results (most seropositive mothers shed CMV in breast milk, which may lead to a false-positive saliva test result in a newly breastfed infant) **[9]**. Refer the Center for disease control and prevention(CDC) website as a resource for information on testing for congenital CMV infection in newborns **[10]**.

b) Antigenemia:

For more than a decade, the antigenemia test has been extensively used to enumerate the CMV virus in blood samples. The usage of monoclonal antibodies to isolate the viral pp65 antigen, a structural late protein formed in blood leukocytes during the early stage of the CMV replication cycle, is necessary for this experiment. Antigenemia is determined by calculating positive leukocyte nuclei in a cytospin sample of 2105 peripheral blood leukocytes using an immunofluorescence test for the CMV matrix phosphoprotein pp65 (PBL) **[2]**. This test is only effective for identifying the virus in leukocytes; positive staining signals in the nuclei of leukocytes show a positive result. The test is quantitative as well as qualitative, and it links well with viremia and clinical illness severity in immunocompromised people **[2]**. The antigenemia assay's drawbacks contain its labor-intensive design, low throughput, and inability to be automated. Subjective bias affects it as well since it necessitates the use of experienced individuals for proper test performance and interpretation of results. The samples must be treated right away (within 6 hours) since any delay would impair the assay's sensitivity. False-negative results are possible, particularly in neutropenic individuals, because the antigenemia test depends on the presence of a satisfactory number of polymorphonuclear leukocytes **[2]**.

2) Serological test:

Based on the presence or absence of CMV IgG, serological testing can be performed to determine if a patient has previously been infected with CMV. Various methods for detecting CMV IgG antibodies have been developed and tested. Some of the techniques used involve complement fixation, enzyme-linked immunosorbent assay (ELISA), anti-complement immunofluorescence, radioimmunoassay, and indirect haemagglutination. The recognition of IgM

antibodies has been used as a marker for acute or current infection. Many altered tests are available; however, enzyme-linked immunosorbent assays (ELISAs), which are based on crude virus arrangements, are the most often used **[2]**.

ELISA:

The most popular serological test for detecting antibodies to CMV is the enzyme-linked immunosorbent assay (ELISA). A positive CMV IgG test indicates that a person has been infected with CMV at some point in their life, but it does not tell when. This relates to persons ≥12 months of age when maternal antibodies are no longer present **[10]**. A CMV IgM-capture ELISA was used to re-evaluate the serological status of CMV IgM determined by the Enzygnost kit (Denka Seiken Co., Ltd., Tokyo, Japan). The manufacturer's procedure was followed for measuring the positive/equivocal/negative samples. Because of their convenience, ELISA kits are increasingly widely utilized; however, Lazzarotto *et al*. found that CMV IgM measures from 8 commercially available ELISA kits had a very low agreement. Siemens AG provided the Enzygnost anti-CMV IgM kit, which uses the traditional indirect method. The samples were measured according to the manufacturer's instructions. To eliminate IgG antibodies, plasma samples were treated with rheumatoid factor (RF)-absorbent (included in the kit). CMV is detected using ELISA in 2 ways: traditional indirect ELISA and IgM-capture ELISAs. **(Figure 1)** Depicts the differences between typical indirect and IgM-capture ELISAs. In a traditional indirect CMV IgM ELISA, anti-CMV IgM in the plasma binds to CMV antigens covering the wells of a 96-well plate. Anti-human IgM in the wells of a 96-well plate of an IgM-capture ELISA captures IgM in plasma samples, whereas anti-CMV IgM is identified by adding a CMV antigen **[11]**.

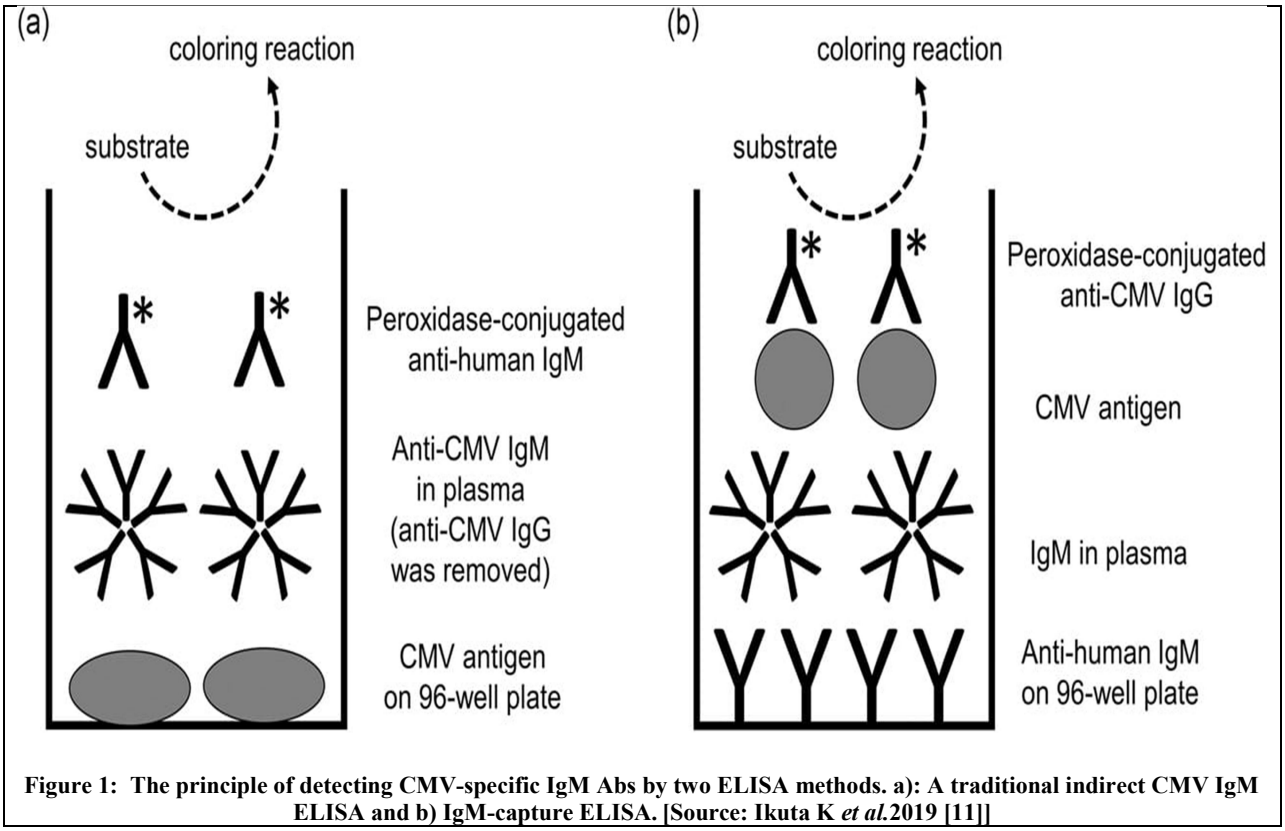

Figure 1: The principle of detecting CMV-specific IgM Abs by two ELISA methods. a): A traditional indirect CMV IgM ELISA and b) IgM-capture ELISA. [Source: Ikuta K *et al.*2019 [11]]

PREGNANT WOMEN

Serological tests are the main tests used to assess pregnant patients for primary CMV infections. Screening for maternal CMV infection occurs mainly after ultrasound results. For women with potential primary CMV infections during pregnancy, identification should be based on either immunoglobulin G (IgG) seroconversion testing or positive CMV IgM and IgG with low IgG avidity testing **[12]**.

INTERPRETATION OF CMV SEROLOGY TEST RESULTS

Table 1: Interpretation of CMV serology test results.

CMV IgG positive	CMV IgM positive
Recommend past infection Only relevant for persons ≥12 months of age (when maternal immunoglobulins are no longer present)	In separation, not helpful to detect primary CMV infection because IgM may also be present throughout recurrent CMV infection.
Paired CMV IgG samples (taken 1-3 months apart)	
It May be used to detect primary infection Seroconversion (initial IgG-negative sample followed over the positive second sample) offers clear evidence for current primary infection	

[Source: CDC, 2020 [9]]

After early serological testing, CMV IgG avidity analysis can help distinguish between primary and recurrent CMV infection in pregnant women.

INTERPRETATION OF CMV IgG AVIDITY TEST RESULTS

Table 2: Interpretation of CMV IgG avidity test results.

Low IgG avidity	Intermediate IgG avidity	High IgG avidity
Recommends primary CMV infection happened within the past 2-4 months	Clinical relevance undetermined	Suggests past infection
During the first trimester, patients should look for consultation with an obstetrician expert with congenital CMV infections; further invasive testing may be warranted	Lower risk of intrauterine transmission	Low risk of intrauterine transmission

[Source: CDC, 2020 [9]]

3) Cell culture:

Conventional cell culture is the classic approach for detecting CMV. Clinical specimens are injected onto human fibroblast cells, cultured, and examined for a duration ranging from 2 to 21 days in this method. CMV has a characteristic cytopathic effect (CPE) in the usual tube cell culture technique, which is characterized by foci of flat, enlarged cells, and the CPE is directly connected to a virus's titer. However, this procedure is slow and takes 2–3 weeks before a negative result can be recorded. The shell vial test is a modified viral culture that uses a centrifugation-amplification process to shorten the time it takes to identify viruses. It makes use of fibroblast cell cultures grown on coverslips in flat-bottom plates. The adsorption of a virus is considerably aided by centrifugation of the specimen onto the cell monolayer, thus enhancing the viral inoculum's infectivity. After 16 hours of incubation, viral antigens could be identified by indirect immunofluorescence using a monoclonal antibody directed at the CMV immediate-early (IE) viral antigen. This approach was modified to work on 96-well microtiter plates, enabling the screening of more samples **[2]**.

4) Immunohistochemistry:

Immunohistochemistry is a technique used to examine tissue or body fluid samples. Slides are generated by centrifuging cells onto a slide or freezing portions of biopsy tissue samples (liver, lung). Then monoclonal or polyclonal antibodies against early CMV antigens are used, which are detected using fluorescently tagged antibodies or enzyme-labeled secondary antibodies that modify the color of the substrate. Fluorescence or light microscopy is used to examine the stained slides. When compared to ordinary histology microscopy, this approach is more sensitive and specific, but it is time-consuming and

requires trained individuals to examine the slides **[2]**. False-negative findings can also arise due to the virus's focused spread **[2]**.

5) Hybrid capture assay:

In an ELISA-style format, the hybrid capture assay employs RNA probes to identify and quantify viral DNA, and the resulting signal is analyzed. Its sensitivity is questioned since it identifies DNA without amplification **[2]**.

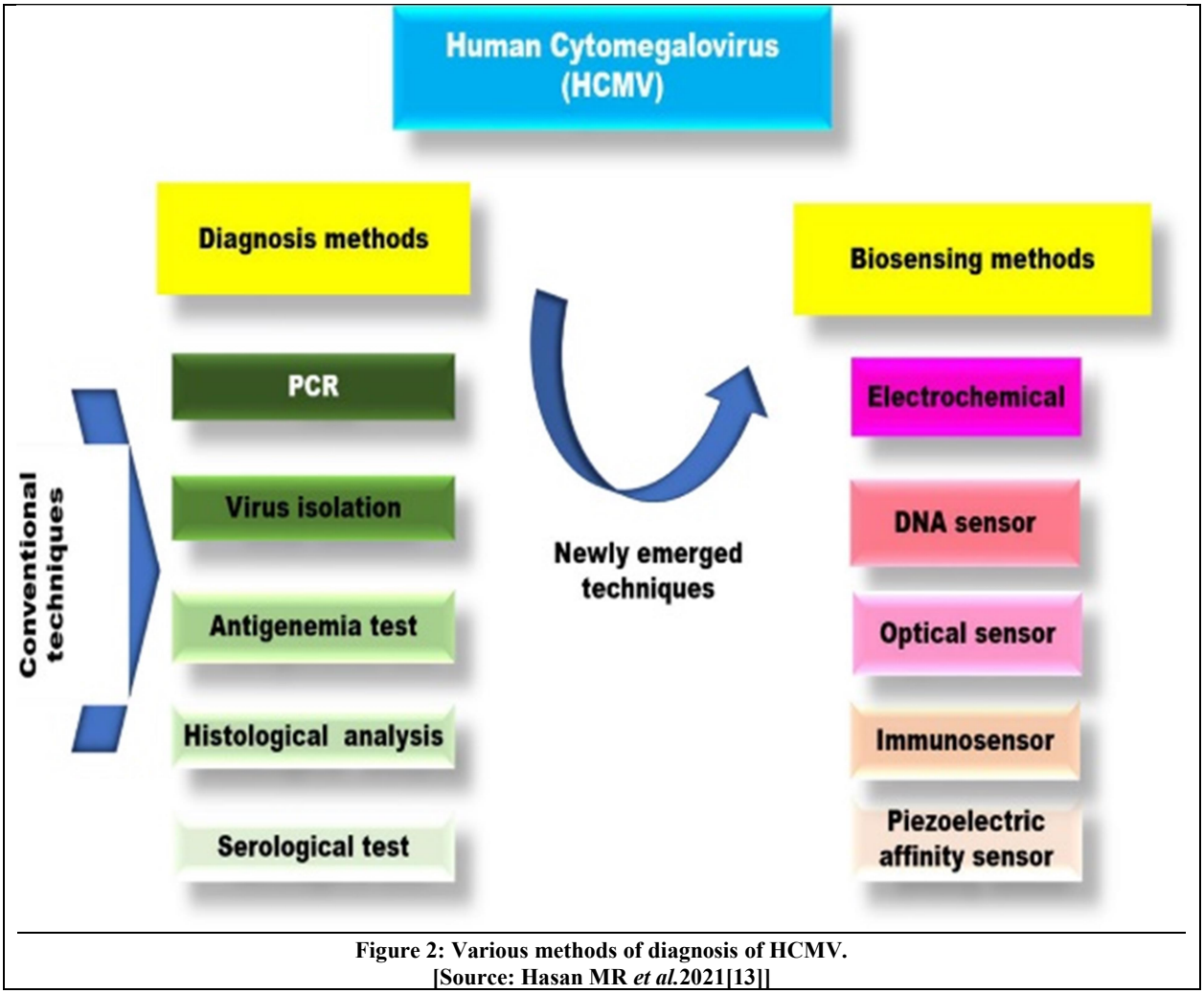

Figure 2: Various methods of diagnosis of HCMV.
[Source: Hasan MR *et al.*2021[13]]

6) Virologic methods:

While neonates with congenital CMV infection shed large amounts of virus, CMV detection in the saliva and urine of newborns is essentially achieved. Traditional tissue culture techniques, as well as the most recent modification of the tube culture technique, centrifugation-enhanced rapid culture techniques (shell vial assay) using monoclonal antibodies to strain for CMV's immediate-early protein, pp72, are considered standard techniques for cCMV

infection detection **[2].** All patients were tested for CMV-IgM antibody and CMV-DNA **[14]**. Rapid culture techniques have been proven to have the same sensitivity and specificity as standard cell culture tests, and results can be obtained in as little as 24 to 36 hours. A 96-well microtiter plate with a monoclonal antibody to the CMV immediate early Ag was shown to be 94.5 percent sensitive and 100 percent specific for detecting CMV in the urine of congenitally infected neonates. This microtiter plate has been designed to work with saliva samples of comparable sensitivity. For the time period, these fast culture techniques provide the standard for detecting cCMV infection **[2]**.

Table 3: Various specimens used for diagnostic tests of CMV infection.

Method	Specimens
Polymerase chain reaction (PCR)	**Blood, Urine, Saliva, Tissue**
Antigenemia	**Blood**
Serology	**Blood**
Cell culture	**Blood, Urine, Saliva**
Immunohistochemistry	**Blood**
Hybrid Capture Assay	**Blood, Tissue**

Source: A Ross S *et al.* 2011[2]

Newly emerged methods:

Biosensors for the detection of HCMV:

The biosensor is a device, which includes a biological identification component, involved in the direct-spatial communication across IUPAC characterization i.e., transduction system. Biosensors may activate as devices that replace a physical and biological occasion into a determinate indication **[15].** It involved a bio-sensing component like-living cells, enzyme tissue that offers selectively, and a transducer, which changes the chemical responses into a processable signal **[13]**. The biosensor is made up of three parts: the bio-mediator, which is a biologically generated substance such as biological sensitive elements, cell receptors, nucleic acids, organelles, microbial tissue, and antibodies. The second element is transducer i.e., piezoelectric, electrochemical, and optical, which converts the signal b the help of signal resultant from the interactions of analytes through the signal processor/allied electronics, allowing for easy monitoring of the visualization method's results. Certain biosensors need a procedure of bio-mediator immobilization to the surface of the sensor (glass, polymer, metal, and other materials) with the help of chemical/physical methods as shown in the **Figure 3**. The limit of detection (LOD), linearity, sensitivity, and specificity of several biosensors for HCMV detection were examined. Each parameter influences the device's functionality. However, the LOD and linearity play a key role in the

manufacture of the device among all measurements. Biosensors should be capable of detecting very small amounts of analyte as well as clinical values **[16]**. The LOD is expressed in concentration units and denotes the smallest amount of analyte in the sample whose concentration can be estimated with sufficient precision and accuracy under particular experimental circumstances. To eliminate misleading negative and positive findings, the biosensor should be extremely specific to the given analyte. The many components of biosensors are visualized in **Figure 3**, which includes bio-recognition elements, transducer types, and amplifiers **[13]**.

Types of biosensors for optimum detection of HCMV-DNA:

1) Electrochemical DNA sensor:

Electrochemical DNA biosensors for optimum detection of HCMV-DNA: DNA is suitable for the application of bio-sensing, as the base-pairing interactions among complementary sequences are robust and accurate. In a standard set-up, immobilization of ssDNA probe sequence inside the detection layer, at which place the base-pairing interactions required DNA target to the surface **[13]**. The repeated, effectively uniform structure of DNA makes its assemblage well-defined upon the surface of identification. At this interface, the 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. However, this recognition occurrence is recorded at last that depends on the transduction of the signal process, whether it is mechanical, electrochemical, or optical. With the aforementioned principle, bio-sensing methods such as microarrays, DNA chips, and DNA sensors tend to attain interest, as they can detect target DNA with higher sensitivity **[13].** The amplified 406-base pair DNA sequence of HCMV has been accurately quantified using an electrochemical DNA identification technique. The aim of this method was HCMV DNA hybridization with Au-NPs (gold nanoparticles) modified oligonucleotide, followed by Au release by acid treatment. As a result, the solubilized AuIII ions were evaluated using ASV with a sandwich-type Screen-printed microband electrode (SPBME) via CV indirectly. Its conjunction of a sensitive Au-III assessment at SPMBE and a large quantity of Au-III emitted by each Au-NPs probe allows it to detect 5 pM of amplified HCMV DNA

segment. However, other advances are required, such as for the production of a compacted, simple to use, easily handled device, or for the aim of working with lesser assay volumes and lesser reaction times to improve sensitivity and low limit of detection **[13]**.

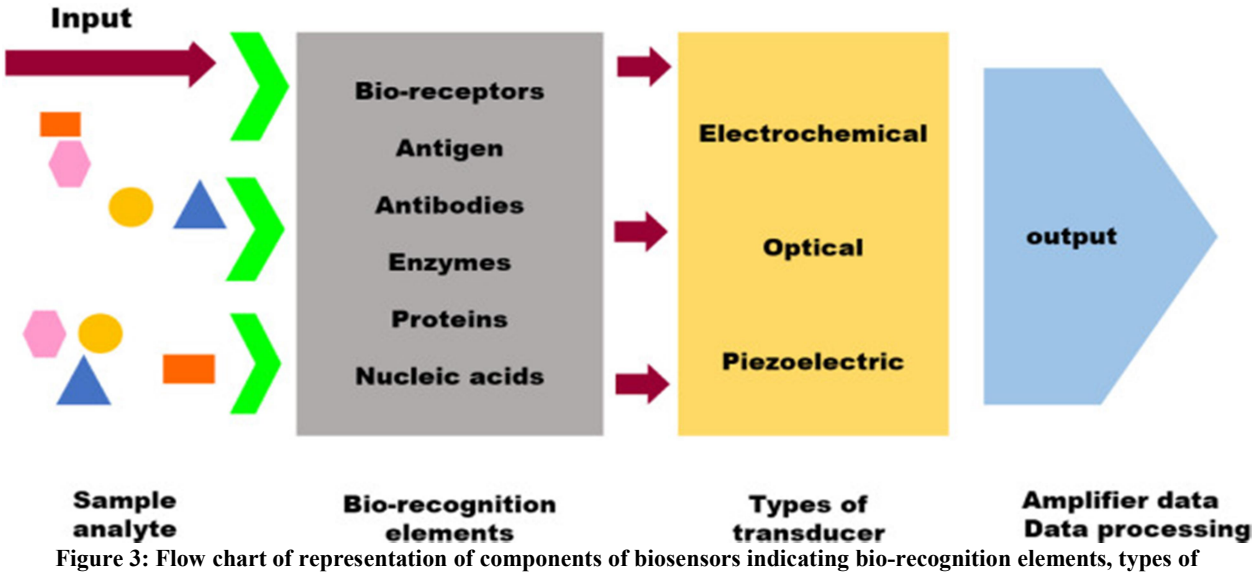

Figure 3: Flow chart of representation of components of biosensors indicating bio-recognition elements, types of transducer, and amplifier.
[Source: Hasan MR *et al.* 2021[13]]

2) Optical biosensor:

Optical sensing of HCMV using surface plasmon resonance technique: The detection of analytes in optical biosensors is aided by optical fibers based on absorption, light scattering, or fluorescence. These biosensors are attracting attention since they allow for multiplexed diagnostics **[17]**. Waveguide devices and optical fibers are employed to increase detection sensitivity in these optical biosensors by enhancing the contact between the sensor surface and the guiding light **(Figure 4)**. The various record wavelengths can be used to identify different kinds of analytes. These biosensors have been used to study a variety of in-vitro phenomena. A fluorescence, phase shift, absorbance, and reflectance optical biosensor recognize and measures changes in several features of a sample. Optical detection can be achieved simply by utilizing fluorescent dyes or fluorescence-labeled aptamers **[18]**. Gietmann *et al*. demonstrated the interaction between the peptide and immobilized HCMV protease using biphasic surfaces of a surface plasmon resonance (SPR) signal-dependent bio-sensor. Because of the reduced km value, an enzyme is immobilized on the surface of the sensor chip by coupling of amines, resulting in active enzymes with high catalytic efficiency as compared to enzymes present in solution. This direct real-time

binding assay provides quantitative and qualitative data on biomolecular interaction by measuring minor changes in chip surface mass. They evaluated the overall significance of the immobilized enzyme's hydrolytic activity for interaction analysis by observing its hydrolytic activity. The use of optical biosensor technology to detect simultaneous binding and conformational changes is predicted, and conformational variations are essential for further characterization of the enzymatic characteristics of HCMV protease and the identification of inhibitors of this enzyme particularly **[13]**.

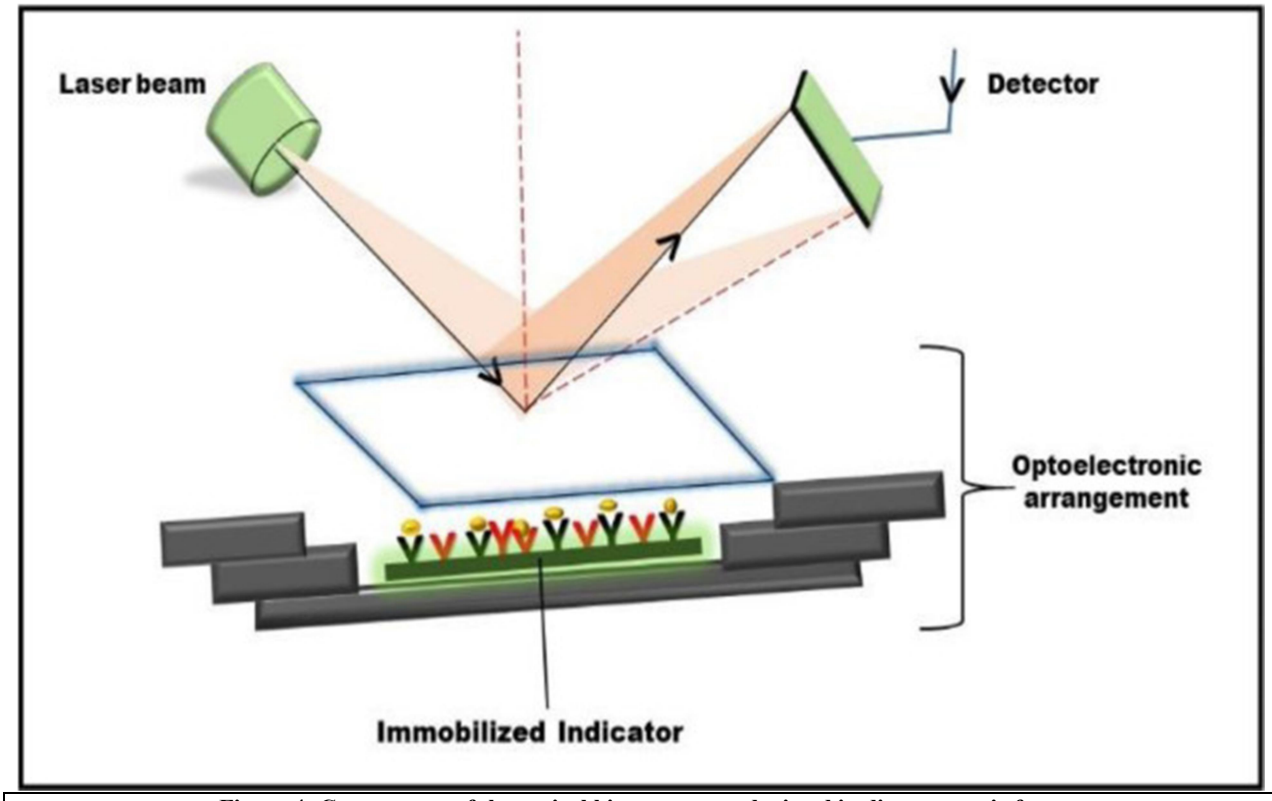

Figure 4: Components of the optical biosensors are depicted in diagrammatic form.
[Source: Hasan MR *et al.* 2021[13]]

3. Piezoelectric affinity sensor:

HCMV underlining strand displacement amplification technique using a piezoelectric affinity sensor: Susmel *et al*. developed a Piezoelectric affinity sensor to detect the HCMV IgB epitope **[13]**. Antibodies immobilized on the Au electrode were used in the sensor. The created biosensor was disposable, using immunological fluorescence on a functionalized surface as the detecting mechanism. This method has a low sensitivity, which means it can only be used with samples with low viral loads. Because of the Ab/Ag interaction, this method has the advantages of being affordable, quick, and extremely specific

[19]. Chen and colleagues developed strand displacement amplification (SDA). It's a well-designed nucleic-acid amplification technology that can work at any temperature. They created a reliable liquid phase identification system in which the crystal oscillator plate was fixed using a simple screw-a-threaded clamping method, and they successfully integrated the innovative sensor system into the real-time HCMV SDA monitoring system **[20]**.

4. Immunosensor:

Immunosensor with electrochemical and optical transduction for HCMV detection: Due to their high-affinity interactions between antigens and antibodies, immuno-sensors are appealing diagnostic instruments that are widely used in biological research **[20]**. Electrochemical immunosensors combine antibody-antigen detection specificity with exemplary electrochemical transduction characteristics connected to high specificity, speed of analysis, and compatibility with mobile devices. By combining the advantages of biosensors with the challenges of HCMV detection, particularly in human samples, a simple and disposable electrochemical immunosensor was developed to detect HCMV Glycoprotein-B in urine samples **[13]**. Glycoprotein-B (gB) was chosen as the electrochemical immunosensor's antigen. Because gB is the most common Ag in the HCMV envelope, nearly all infected people generate antibodies against it. HCMV gB might be a significant factor to consider when developing HCMV detection test. Using the above-mentioned method, a method was presented that was dependent on the immunoassay, i.e., sandwich-based type with secondary antibody (Abs) tagged with Au NPs, that allows Ag-NPs (silver nanoparticles) precipitation, resulting in increased immunosensor sensitivity. Glycoprotein-B was quantified by electrochemical stripping-analysis of Ag-NPs and captured on an immuno-sensor using catalysis with nano-gold labels **[21]**.

DISCUSSION

In this review, we strive to provide information on CMV infection diagnosis and different stages. In the below table method, advantages and disadvantages of different diagnosis techniques are discussed.

Table 4: Summary of the method, advantages, and disadvantages of conventional methods of HCMV

	Method	Advantages	Disadvantages
Polymerase chain reaction(PCR)	**Recognition of the nucleic acid target sequence.**	**Effective at detecting CMV DNA in a variety of samples, including plasma, blood, Bronchoalveolar lavage fluid, and cerebrospinal fluid.**	**Clinical specificity suffers as analytical sensitivity increases.**

Antigenemia test	**The quantitative analysis indicates a viral load that benefits in monitoring patients before and after treatment.**	**Useful for evaluating the possibility of ailment development.**	**It is labor-intensive and does not accept automation.**
Serological test	**Identification of CMV IgG and IgM.**	**It is very fast so that a result can be obtainable within the same day.**	**Gives reasonably accurate results within a minute.**

[Source: Hasan MR *et al.* 2021[13]]

In the below table different biosensors which detect the HCMV are described.

Table 5: Summary of different biosensors to detect the CMV.

Biosensor	**Electrochemical DNA sensor**	**Optical -biosensor**	**Piezoelectric biosensor**
Principle	**Based on amplicon sequence variant(ASV) on SPMBE**	**SPR dependent method**	**Based on the strand displacement amplification technique**
Markers detected	**HCMV DNA**	**Human Cytomegalovirus protease and peptide interactions.**	**The nucleic acid of CMV**
Advantages	**Fast and low cost**	**Reusable, label-free, high sensitivity, simple**	**Highly sensitive, less time-consuming, fast**
Disadvantages	**The highly buffered solution may interfere**	**Low selectivity, non-specific binding to surfaces, requires strict observance.**	**Usually for dynamic measurement only, high-temperature sensitivity.**

[Source: Hasan MR *et al.* 2021[13]]

Future Directions:

Our understanding of the natural history and pathogenesis of congenital CMV infection, as well as the importance of antiviral therapy for congenitally infected children, is constantly improving. It is predicted that future efforts to develop and standardize molecular diagnostic technologies will result in the availability of reliable, quick, and easy procedures for routine clinical usage. In addition, there is rising interest in evaluating the viability of integrating a newborn CMV screening program with a universal newborn hearing screening. DBS PCR tests have not been demonstrated to have sufficient sensitivity for identifying most newborns with congenital CMV infection, which is quite discouraging. However, the development of saliva PCR assays has the potential to adapt these approaches to screen a large number of infants for congenital CMV infection in a high-throughput manner. Furthermore, the method of measuring virus burden in saliva specimens from newborns with asymptomatic congenital CMV infection using saliva PCR assays could provide a method of identifying at-risk infants early in life, allowing for more efficient available resources by focusing on at-risk children for follow-up and monitoring **[2]**. The availability of effective treatments will add to the motivation to develop better diagnostic tests and algorithms. To manage the societal

burdens caused by HCMV, large-scale worldwide clinical and epidemiologic interactions will be required. **[22]**

CONCLUSION

This review article's purpose is to provide current information on CMV diagnosis. In the absence of active theragnostic involvement, CMV-related illness continues to progress. As a result, sensitive, specific, and quick detection of current CMV infection is a common requirement. The current review article focuses on the impact of biosensors and their sub-classes in detecting HCMV. With a better understanding of HCMV diagnostics, it can be determined that electrochemical techniques are the best option for all criteria. HCMV may be diagnosed using electrochemical techniques, which are selective, sensitive, and quick. In addition, several tiny devices are used for electrochemical sensing. Currently, paper-based biosensors have revolutionized sensing, as they provide several advantages such as cost-effectiveness, speed of response, facile approach, and minimum sample requirements.

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