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Monoclonal antibodies for diagnosis of viral diseases: a comprehensive review

Abhishek Kasana, Aishwarya Dwivedi, Vaishali Verma*

Department of Biotechnology, School of Engineering and Applied Sciences, Bennett University, Greater Noida, Uttar Pradesh, India.

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ABSTRACT

Viruses cause infectious diseases in humans, ranging from mild to life-threatening. Early and accurate diagnosis is essential for timely treatment and prevention of complications. Diagnostic methods typically rely on detecting viral genetic material, proteins, or the host's immune response, depending on the infection stage. Monoclonal antibodies, known for their specificity and high affinity, are widely used for diagnosing viral infections by enabling precise and sensitive detection of viral proteins. This review explores the applications of monoclonal antibody-based methods for detecting viral infections, emphasizing their pivotal role as sensitive detection reagents. These methods are crucial for developing robust immunodiagnostic assays capable of diagnosing various viral diseases in human, animal, and plant hosts, with special emphasis on SARS-CoV-2. The review also explores various assays that employ monoclonal antibodies for diagnostic purposes and the technologies used for their production. Understanding the principles and applications of monoclonal antibodies in diagnosing viral diseases is essential for implementing effective public health interventions and preventing future pandemics.

1. INTRODUCTION

Viral diseases are illnesses caused by viruses that replicate inside the living cells of other organisms (referred to as the host). Viruses cannot replicate independently outside of a host cell. Consequently, they must infect host cells and take over their machinery to generate more virus particles. Viral pathogens have significantly threatened human safety during the past few decades. Viral diseases can cause a range of symptoms depending on the virus, from mild respiratory symptoms to life-threatening conditions. Such diseases are often contagious and have the potential to rapidly disseminate among populations, making prevention and control efforts crucial for public health. Consequently, rapid and precise diagnostic procedures are essential for the timely identification of viruses. This enables the prompt administration of appropriate treatments, thereby improving clinical outcomes and aiding in the control of infection spread [1–3].An ideal detection method must meet several key criteria: high specificity, quick turnaround time, operational simplicity (no need for time-consuming sampling procedures or specialized equipment), and affordability. Diagnostic approaches for viral diseases can be broadly classified into nucleic acid-based methods and protein-based methods. The nucleic acid-based methods rely on detecting the viral genome, whereas protein-based methods focus on detecting virus-specific proteins (antigen-detection methods) or antibodies produced by the host in response to the virus (serological/antibody-detection methods) [4]. Nucleic acid-based methods necessitate the design of specific primer sets to amplify virus-specific sequences, offering high sensitivity. However, these methods require costly chemicals and equipment, such as polymerase chain reaction (PCR) machines, for amplification reactions. In addition, they are susceptible to contamination during sample handling, necessitating skilled personnel. Protein-based antigen detection methods use target-specific antibodies to detect viral proteins [5]. Due to their high specificity towards targets; antibody-based detection tools are a viable diagnostic option for diagnosing a wide range of pathogens . These methods can be highly sensitive, depending on antibody affinity, and can detect proteins during the early stages of infection [6]. Furthermore, antigen detection tests are highly stable and have a longer shelf life [7]. Antibody-based assays can be formatted into point-of-care assays, which are rapid (completed in ~30 min), easy to perform, and generally do not require specialized laboratory equipment or skilled personnel. The COVID-19 pandemic underscored the significance of rapid antigen-based lateral flow assays (LFAs), which proved invaluable for detecting SARS-CoV-2 and implementing containment measures, demonstrating the utility of protein-based assays in viral disease diagnosis [8].

Antibodies are classified into monoclonal and polyclonal types [9]. Monoclonal antibodies are derived from a single clone of plasma B cells [9]. In contrast, polyclonal antibodies are obtained from multiple clones of plasma B cells. Monoclonal antibodies exhibit greater specificity, consistency, and reliability than polyclonal

*Corresponding Author:
Vaishali Verma,
Department of Biotechnology, Bennett University, Plot 8-11, Tech Zone II, Greater Noida, Uttar Pradesh.
E-mail: Vaishali.verma @ bennett.edu.in

antibodies, with reduced cross-reactivity against other antigens [10]. Various diagnostic techniques utilize monoclonal antibodies, namely enzyme-linked immunosorbent assay (ELISA) [11], Western blotting [12], radioimmunoassay (RIA), and LFA [13]. This review highlights the pivotal role of monoclonal antibodies in developing highly specific, sensitive, and rapid immunodiagnostic tests for detecting viral pathogens. We reviewed existing literature to examine the applications of specific monoclonal antibodies against viral targets for diagnostic tests, with special emphasis on SARS-CoV-2. Additionally, we discussed strategies for their production for diagnostic applications along with the immunoassays that employ them.

2. ASSAYS USED FOR THE DETECTION OF DISEASES USING MONOCLONAL ANTIBODIES

Currently, antibody-based detection of target proteins plays a crucial role in disease diagnostics. These assays rely on non-covalent antibody-antigen interactions, in which antibodies specifically recognize the target antigen, thereby confirming the presence of pathogens. The sensitivity and specificity of these techniques depend on the strength of antibody binding to its antigen [14]. The most widely used antibody-based antigen detection formats include ELISA, LFA, Western Blot, dot blots, and immunohistochemistry (IHC). These assays offer distinct advantages in sensitivity and ease of use, catering to diverse diagnostic needs [Table 1 and Figure 1]. Both polyclonal and monoclonal antibodies are utilized to develop immunodiagnostic tests [15]. The scalable production of recombinant monoclonal antibodies ensures a reliable and cost-effective supply for diagnostic applications, making them highly attractive for commercialization.

ELISA, a specific and sensitive biochemical test, facilitates analyte detection and quantitative analysis without requiring specialized equipment [16]. It is based on the principle of antigen-antibody binding, where the protein of interest is immobilized, and a specific antibody binds to it. Detection of this binding is achieved using a secondary antibody coupled with an enzyme, resulting in a color change upon reaction with a chromogenic substrate [17]. Fluorogenic or chemiluminescent substrates provide enhanced sensitivity for quantitative measurements [17]. Four major types of ELISA are commonly employed for the detection of proteins: direct, indirect, sandwich, and competition ELISA. In direct ELISA, the surface of the plate is coated with either the antibody or antigen present in the sample. Detection is achieved through an enzyme attached to either the antibody or antigen, facilitating accurate measurement [18,19]. It finds applications in the detection of various pathogens, including viruses, such as SARS-CoV-2 [20,21], Porcine Circovirus 3 (PCV3) [22], Cache Valley virus (CVV) [23], Pseudorabies virus, etc. [24]. Indirect ELISA is a two-step detection assay. Initially, the target-specific primary antibody attaches to the antigen, and subsequently, an enzyme-linked secondary antibody is introduced to detect and bind to the primary antibody [25–27]. Indirect ELISA is mostly used for serological testing, which involves the detection of the host's immune response against the pathogen. It has shown high sensitivity and specificity for detecting IgG and IgM antibodies against Crimean-Congo hemorrhagic fever (CCHF) in acute and convalescent human sera [28]. This assay also finds applications in detecting SARS-CoV-2 antibodies in cats [29]. Sandwich ELISA, by immobilizing antigens between capture and detector antibodies, offers increased sensitivity and specificity in detecting target antigens. It finds applications in the detection of the Ebola virus [30] and Citrus leprosis virus C2 [31]. Competition ELISA assesses antigen-antibody interactions by competitive binding assays [32]. This assay finds various applications in the diagnosis of different viral diseases, such as porcine epidemic diarrhea virus (PEDV) [33] and Goose astrovirus (GAstV) [34].

Western blotting, or immunoblotting, identifies proteins separated by gel electrophoresis using antibody detection [35]. Multiple studies have reported the use of this technique in the characterization of antibodies developed for the detection of viral diseases [21,22,36–38]. Immunohistochemistry (IHC) utilizes antibodies to determine the location of proteins or antigens in tissues through visualization of antigen-antibody interactions in tissue samples using colored enzyme substrates or fluorescent dyes [39]. Researchers use this technology to study virus-infected tissue samples [40,41].

Lateral Flow Assays (LFA) first reported in the 1960s, revolutionized diagnostic accessibility by employing monoclonal antibodies for rapid and straightforward antigen detection [42]. LFAs detect viral antigens or antibodies in diverse samples (e.g., blood, saliva) via specific immobilized monoclonal antibodies on a nitrocellulose membrane. Multiple studies have reported the development of LFA-based methods for detecting viral antigens [36,43,44]. Advanced LFAs integrated with artificial intelligence-based reading technologies have been employed to enhance detection sensitivity for viruses such as SARS-CoV-2, achieving up to 86.2% sensitivity compared to 71.4% when assessed by human eye. This advancement underscores the effectiveness of combining traditional diagnostic methods with cutting-edge technology to improve accuracy and reliability in virus detection [45].

Table 1: Summary of the properties of key immunodiagnostic techniques.

Detection Method	Sensitivity	Specificity	Ease of Use	Advantages	Disadvantages
ELISA	High (Typically, >10-100 pg; depends on substrate and antibodies used)	Highly specific	Requires trained manpower and is labor intensive	Highly sensitive and specific, faster than Western blot	Time consuming (at least 2-3 h), requires specialized equipment, expensive
Western blot	High (Typically, >100 pg; depends on substrate, membrane, and antibodies used)	Highly specific	Requires trained manpower and is labor intensive	Highly sensitive and specific	Time consuming (at least 4-6 h), requires specialized equipment, expensive
Lateral flow assay	Lower as compared to ELISA and Western blot (Typically, >10 ng; depends on membrane, and antibodies used)	Highly specific	Easy to perform and doesn't require training	Rapid results (15-20 min), easy to use, economical, portable	Low sensitivity as compared to ELISA and Western blot

Table 2: Comparison of techniques to produce monoclonal antibodies for diagnostic applications.

Technology	Principle	Advantages	Limitations
Hybridoma technology	Animals are immunized with the target antigen, followed by the fusion of immortal myeloma cells with B lymphocytes [77].	Robust and has been used for the discovery of large number of antibodies for different applications [78]. Once clones are established, monoclonal antibody production becomes straightforward. Preserves original pairing of antibody chains present in B cells [79]. Hybridoma antibodies can be used directly and can be cryopreserved until the subsequent use for an indefinite time period [80].	Non-availability of fusion partners limits its use and multi-species applicability [81]. Requires a purified antigen for immunization. In some cases, it may be difficult to purify the antigen. Efficacy depends on animal health and immunization efficiency. High risk of contamination [82]. Majority of B cells are lost during fusion, which leads to a loss of efficiency [9].
Phage display technology	Antibodies derived from naïve or immunized B cell sources are displayed on the surface of bacteriophages [83]. A library of these phage particles is created, and each displays a unique antibody fragment on its surface. It can be used in the isolation of specific binders.	Comparatively faster and more optimized. There is no necessity to immunize animals for phage display when using naïve libraries. Sufficient depth of coverage for finding antigen-specific antibodies compared to other methods [84].	Good clones may get missed due to poor RNA recovery or loss of DNA during library construction [84]. Requires high transformation efficiency during preparation of large libraries.

3. TECHNOLOGIES FOR THE DEVELOPMENT OF MONOCLONAL ANTIBODIES FOR DIAGNOSTIC APPLICATIONS

As elaborated above, monoclonal antibodies play a crucial role in disease diagnosis. Several technologies are available for developing monoclonal antibodies for diagnostic and therapeutic applications [Figure 2]. These techniques include hybridoma technology, phage display, single B cell cloning technology, the use of humanized animals (such as mice) encoding human antibody genes, next-generation sequencing-based technology, and computational/AI-based technologies.

The hybridoma method developed by G. Köhler and C. Milstein [46] is a time-tested technique to obtain monoclonal antibodies (2). Once desirable antibody-secreting hybridoma clones are selected, they are cultured on a large scale to produce antibodies in desired quantities [47]. Monoclonal antibodies developed using this technique are mostly preferred among all the available methods because the isolated antibodies are highly specific to the target antigen [9]. Also, this technique is more convenient and cost-effective than other techniques [48]. This technique has expanded the antibody discovery and production scope across various applications. Multiple researchers have reported the development of monoclonal antibodies against viral antigens using hybridoma technology [21,36,49,50].

George Smith invented the phage display technology in 1985 and revolutionized the process of discovery of antibodies for diagnostic, therapeutic, and basic research applications. This technology is based on the ability of bacteriophages to display foreign proteins, such as antibody fragments, on their surface. At the same time, they encode the gene inside the phage particle. Usually, M13 filamentous bacteriophage is used as the viral vector for displaying antibody fragments on its surface. The process involves the construction of a phage display library, which is a collection of millions to billions of different phage particles, each displaying a unique antibody fragment on its surface [Table 2]. Typically, such a library is generated by amplification of genes encoding antibody variable heavy and light chain domains from the B cells isolated from immunized (animals such as mice or convalescent humans) or non-immunized sources, followed by their PCR-based assembly and cloning in frame with the coat proteins (mostly g3 protein) of M13 bacteriophage. The resulting phage library is then subjected to biopanning, to eliminate non-binding or low-affinity phages, and recover target specific phages. After several rounds of biopanning, the individual phage clones displaying the desired antibody fragments are isolated, and the DNA encoding the antibody fragments is sequenced. After determining the sequences, the variable genes of antibodies can be subcloned into different expression systems. Recombinant monoclonal antibodies developed using phage display offer several advantages over traditional hybridoma-derived antibodies [Table 2]. These include improved stability, consistent quality, and the ability to engineer desired properties like higher affinity or specificity [51].

Typically, single B cell cloning technology involves the isolation of B cells from vaccinated or convalescent humans, followed by the sorting and culturing of B cells as single cells, screening of B cell supernatant for antibody specificity against the target, and cloning of antibody genes into mammalian expression vectors to produce full-length monoclonal antibodies [52,53]. This technology retains the inherent pairing of light and heavy chains expressed by a B cell and has been successfully used to isolate fully human antibodies as therapeutics for the treatment of a wide range of viral diseases, including SARS-CoV-2 [54]. Researchers have also developed humanized mice in which the locus encoding mouse antibody genes is replaced with human antibody genes. Consequently, upon immunization, these mice yield human antibodies instead of mouse antibodies, which can be directly employed for therapeutic applications without requiring humanization [55]. Deep sequencing of B cell repertoires using next generation sequencing (NGS) technologies has also enabled the identification of antibodies against desired targets [56]. NGS can also be combined with other antibody discovery technologies, such as phage display, to discover antibody leads [57]. Recently, artificial intelligence (AI)-based methods have also become available to predict antibody sequences specific to a target *in silico* [58]. AI-based methods are actively being explored for discovering new antibodies and improving the properties of existing antibodies [59].

While all these technologies have been successfully employed for the production of monoclonal antibodies for therapeutic and diagnostic applications, hybridoma technology and phage display technology

Table 3: Studies reporting the development of monoclonal antibodies for the detection of SARS-CoV-2.

S. No.	Immunogen	Technology used for antibody discovery	Methods used for antibody characterization	Detection limit	References
1.	Spike protein ECD	Phage Display	Indirect ELISA, SPR, Competitive ELISA, and Sandwich ELISA	43.2 ng/mL with sandwich ELISA	[20]
2.	Spike protein (Full-length)	Hybridoma Technology	ELISA, Dot Blot, and LFA	781 PFU with sandwich ELISA	[49]
3.	Multiple SARS-CoV-2 proteins	Phage Display	ELISA, BLI, Western Blot, and IFA	Not reported	[37]
4.	UVC-inactivated SARS-CoV-2QLD02	Hybridoma Technology	IHC, Indirect IFA, ELISA, Western Blot, and LFA	100 ng/mL using LFA	[36]
5.	Nucleocapsid and Spike protein (S1)	Hybridoma Technology	ELISA, Flow cytometry, and LFA	4.89–9.06 ng/mL with S1 and 0.76–6.95 ng/mL with nucleocapsid-based LFA	[43]
6.	Nucleocapsid protein	Hybridoma Technology	ELISA, BLI, and LFA	240 ng/mL using LFA	[44]
7.	Truncated Nucleocapsid protein	Hybridoma Technology	BLI, Immunoblotting, IFA, IHC, sandwich ELISA and LFA	$6.3x10^4$ virus copies/mL or 6.25 pg/mL of recombinant protein using LFA	[50]
8.	Nucleocapsid protein	Phage Display	ELISA, Western blot, Dot blot and BLI	$2.5x10^4$ PFU per reaction	[60]
9.	Truncated Nucleocapsid protein	Hybridoma Technology	Indirect ELISA, Immunofluorescence Analysis, Western Blot, Sandwich ELISA	5500 PFU/mL using sandwich ELISA	[21]

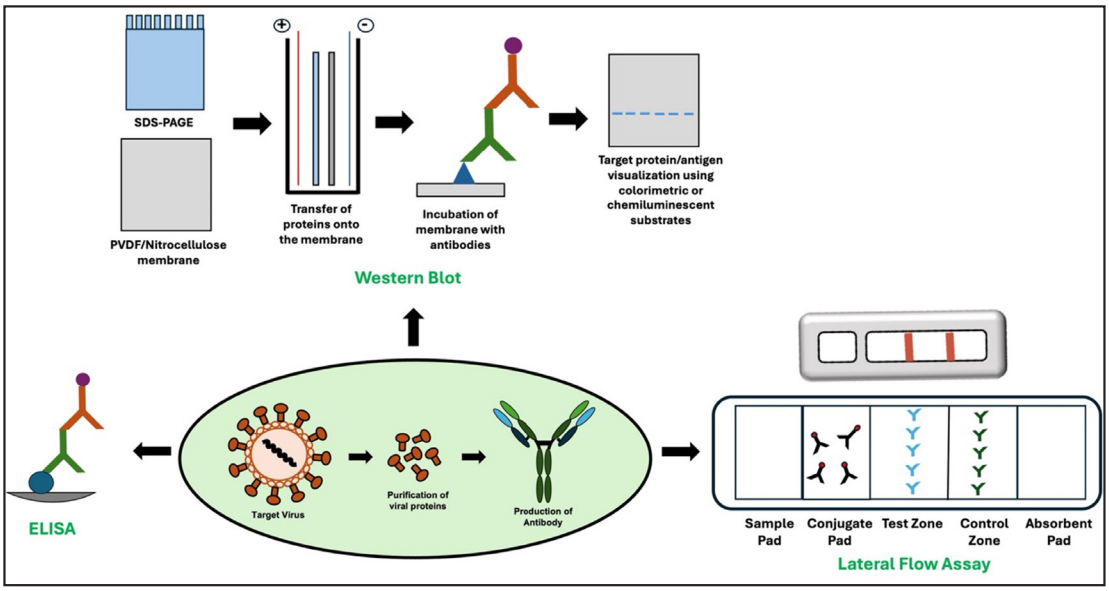

Figure 1: Applications of antibodies specific to viral targets in immunodiagnostic assays.

are the most commonly used techniques for developing monoclonal antibodies for diagnostic applications. Both techniques facilitate the isolation of antibodies with desired characteristics, such as high affinity, specificity, and tailored cross-reactivity.

4. DEVELOPMENT OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF SARS-COV-2

During the COVID-19 pandemic, there was significant emphasis on developing monoclonal antibodies against SARS-CoV-2 for both diagnostic and therapeutic purposes. SARS-CoV-2, a positive-strand virus, encodes multiple structural and non-structural proteins. Several research groups reported successful efforts in developing monoclonal antibodies aimed at rapid detection of SARS-CoV-2 [Table 3].

Kim et al., reported a sandwich ELISA-based method for detecting SARS CoV-2 [20]. Using phage display technology, they developed mouse monoclonal antibodies against the extracellular domain of spike protein (SARS-CoV-2 S-ECD). Two monoclonal antibodies (K104.1 and K104.2) were isolated from a synthetic phage display library and were characterized using phage ELISA, surface plasmon resonance (SPR), competition ELISA, and sandwich assay. These were used as

Table 4: Studies reporting the development of monoclonal antibodies for detecting viruses other than SARS-CoV-2.

S. No.	Virus	Immunogen	Method Used for Antibody Characterization	Detection Limit	References
1.	Porcine Circovirus 3	Capsid protein	Indirect ELISA, Western blot, IFA, Dot blot, EB-ELISA	Not reported	[22]
2.	Varicella-zoster virus (VZV)	Glycoprotein E (gE)	IFA, immunoperoxidase monolayer assay, ELISA, Western Blot, LFA	30 ng/mL of purified gE using LFA	[61]
3.	Pseudorabies virus	Inactivated whole virus protein	Indirect ELISA, IFA, Western Blot	Not reported	[24]
4.	Classical swine fever virus	Envelope protein E2	Indirect ELISA, Western blot, competition ELISA	Not reported	[71]
5.	Cache Valley virus (CVV)	Nucleoprotein	ELISA, Western blot, IFA, MAC-ELISA	Not reported	[23]
6.	Hepatitis E virus (HEV)	HEV ORF3 protein	Indirect ELISA, Western blot, Competitive ELISA	Not reported	[70]
7.	Zika virus	Envelope protein	ELISA, LFA	33 µg/mL of recombinant E protein and 6.3×106 PFU/mL of ZIKV using LFA	[62]
8.	H3 influenza A virus	Inactive H3 virus	Sandwich ELISA, Western Blot	$>10^6$ virus dilution using Sandwich ELISA; Amount not reported	[64]
9.	Chikungunya Virus	Envelope 2 protein	ELISA, Immunoblotting	0.7 µg/mL inactivated CHIKV using indirect ELISA	[68]
10.	MERS-CoV	Spike protein	Sandwich ELISA	5.89 ng/mL of MERS-CoV S protein using Sandwich ELISA	[65]
11.	Zaire Ebola virus	Zaire Ebola virus glycoprotein	SDS-PAGE, Western blot, Dot blot, Indirect, IFA, sandwich ELISA	3.6 ng/mL rGPdTM with Sandwich ELISA	[30]
12.	Dengue Virus (DENV-4)	NS1	Western Blot, Sandwich ELISA	32.5 ng/mL of NS1 protein with Sandwich ELISA	[66]
13.	Zucchini yellow mosaic virus	ZYMV virion	ACP-ELISA, dot-ELISA, tissue dot-ELISA, DAS-ELISA and IC-RT-PCR	ACP-ELISA (1:163840*), dot-ELISA (1:2560*), DAS-ELISA (1:327680*), IC-RT-PCR (1:1310720*) * Dilution of ZYMV-infected crude extracts	[72]
14.	Yellow fever virus	Recombinant Envelope protein and YF vaccine virus 17D	Indirect ELISA, Western blot, IFA, and sandwich ELISA	1 x 10^3 FFU/mL of YFV 17D or 2ng/well of recombinant envelop protein using sandwich ELISA	[67]
15.	MERS-CoV	Truncated Nucleocapsid Protein	Immunoprecipitation assay, Sandwich ELISA, LFA	0.5 ng of recombinant protein and 3 x 10^6 copies of MERS-CoV virions using LFA	[63]
16.	Chikungunya Virus	Capsid protein	ELISA, Western blot, IFA, and IHC	Not reported	[40]
17.	Tembusu Virus	Envelope protein	Western Blot, Sandwich ELISA	Not reported	[69]
18.	SARS-CoV	Spike protein subunit S1	SDS-PAGE, Western Blot, Indirect ELISA, Sandwich ELISA	0.019 µg/mL of S1 protein using Sandwich ELISA	[38]
19.	Seneca Valley virus	Binary ethylenimine (BEI) inactivated SVV	Dot blot, IHC, competitive ELISA	Not reported	[41]

capture and detection antibodies to develop sandwich ELISA-based immunoassays. The limit of detection (LOD) was found to be 43.2 ng/mL for the extracellular domain of the spike protein. Since this pair targeted a relatively more conserved region of spike protein, it also allowed detection of the spike proteins of other SARS-CoV-2 variants [20]. Mariotti et al., explored the development of LFA-based methods for detecting SARS-CoV-2 [49]. They developed mouse monoclonal antibodies using hybridoma technology against the full-length spike protein of the SARS-CoV-2 and characterized 13 antibodies using ELISA and Western blot. Epitope mapping revealed that nine

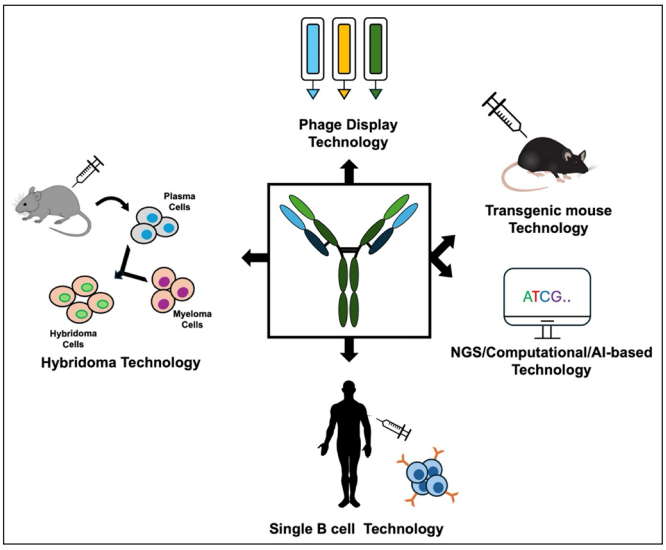

Figure 2: Technologies used for the production of monoclonal antibodies.

antibodies were specific to the S1 domain, while four recognized the S2 domain. The antibodies were also characterized using dot blot and LFA. They were found to be highly specific and could detect viruses produced in the lab and in the biological samples. The best pair of antibodies, S71-S79, allowed the detection of 781 plaque-forming units (PFU) using sandwich ELISA.

In addition to spike protein, other proteins have also been explored for the development of diagnostic tests against SARS-CoV-2. Morgan et al., developed mouse monoclonal antibodies against the UVC-inactivated whole virus (SARS-CoV-2QLD02) using hybridoma technology [36]. Two antibodies, SCV2-6A11 and SCV2-7E9, were used to develop LFA, which allowed the detection of >100 ng of the recombinant spike protein. One antibody, SCV2-1E8, allowed the detection of multiple virus variants and worked efficiently in ELISA, Western blot, and immunofluorescence assay (IFA) applications [36]. Mishra et al., reported the development of 18 monoclonal antibodies against 9 different SARS-CoV-2 proteins using a synthetic Fab-displayed phage library [37]. The antibodies were characterized by ELISA, Biolayer Interferometry (BLI), Western Blot, and IFA, and antibodies 15884 and 15887 were useful for studying subcellular locations of SARS-CoV-2 viral proteins using IFA. The antibodies were found to target different epitopes, allowing their use for the development of multiple assays for detecting SARS-CoV-2. Salcedo et al., developed mouse monoclonal antibodies against both nucleocapsid and spike protein (S1) of SARS-CoV-2 using hybridoma technology [43]. B cells derived from the spleen and lymph nodes of immunized mice were used for spike protein (S1), whereas lymph nodes were used for nucleocapsid protein. Eleven antibody pairs (7 for S1 and 4 for nucleocapsid protein) were tested using LFA, and the LOD ranged from 4.89–9.06 ng/mL for recombinant S1 protein and 0.76–6.95 ng/mL for nucleocapsid proteins. Further, the nucleocapsid-based LFA developed using antibodies 1 and 453 showed 84.6% sensitivity and 100% specificity using patient nasal samples. Xie et al., developed an LFA-based test for detecting SARS-CoV-2 virus using mouse monoclonal antibodies against nucleocapsid protein [44]. The antibodies were characterized using a paired screening by a double sandwich method using BLI, and three antibodies, namely N3, N10, and N15, were selected to develop LFA. The N10 (gold conjugated) and N15 (test line) pair showed the maximum sensitivity and allowed the detection of 240 ng/mL of recombinant nucleocapsid. The LFA also detected the recombinant nucleocapsid produced by the Delta (B.1.617.2) variant of the SARS-CoV-2.

Yamaoka et al., used truncated nucleocapsid protein (121–419 amino acids) for the development of mouse monoclonal antibodies [50]. They established 144 stable hybridomas and characterized them using indirect ELISA, AlphaScreen, and BLI. The affinity of antibodies was found to be in the sub-nanomolar range for three antibody clones. These clones allowed detection of nucleocapsid protein in the lysates of virus infected cells using immunoblot. Antibodies 7 and 98 also worked in IFA, whereas 98 also allowed the detection of virus in the paraffin-embedded lung tissue of an infected patient using IHC. Antigen-capture ELISA using antibodies 9 (capture) and 98 (detector) allowed highly sensitive detection of as low as 3.2 pg/mL of recombinant nucleocapsid protein or $3.3x10^4$ copies/mL of the inactivated virus. LFA developed using the optimized pair was found to be highly specific to SARS-CoV-2 and allowed detection of 6.25 pg/mL of recombinant nucleocapsid protein or $6.3x10^4$ copies/mL of the inactivated virus. Kim et al., developed chicken scFv antibodies against full-length nucleocapsid protein using phage display technology [60]. Purified nucleocapsid protein was panned against a chicken naïve phage library, and during selections, clones reactive to SARS-CoV and MERS-CoV were eliminated. The SARS-CoV-2 specific scFvs were produced in scFv-Fc format for further characterization. The affinity of four clones was found to be in the nanomolar range. The LOD of selected clones was tested using LFA and a combination of 12H8–12H1 antibodies allowed the detection of as low as 2.5×10^4 virus PFU per reaction with no cross-reactivity to other viruses, namely SARS-Co-V, MERS-CoV, and influenza virus. Terry et al., developed mouse monoclonal antibodies against truncated nucleocapsid protein (133-419 amino acids) of SARS-CoV-2 using hybridoma technology [21]. Two antibodies, mBG17 and mBG86, allowed the detection of virion-derived N protein from 5500 PFU/mL and 55,000 PFU/mL SARS-CoV-2 virions, respectively, with rabbit polyclonal antibody as the capture antibody in sandwich ELISA.

Overall, based on studies describing the development of monoclonal antibodies for detecting SARS-CoV-2, it is evident that hybridoma technology and phage display are the most widely used technologies for producing these antibodies. The primary target proteins for these antibodies are spike protein and nucleocapsid protein [Table 3]. The spike protein is present on the surface of the SARS-CoV-2, while the nucleocapsid protein coats the RNA genome present inside the virus particle. Due to their high copy numbers, both proteins are important targets for developing sensitive detection strategies. Both truncated and full-length versions of these proteins have been employed to generate antibodies. The developed antibodies have been utilized in various assay formats, including ELISA-based methods (such as sandwich and indirect ELISA), LFAs, and IFAs. This variety allows for flexibility in different diagnostic settings and resource availability. The assays have demonstrated high sensitivity, with LOD reported as low as single-digit picogram ranges [50] or detecting as few as 781 plaque-forming units (PFU) of virus [49]. While studies have explored antibodies from human [37] and chicken origin [60] for diagnostic development, the mouse remains the most widely used host for producing monoclonal antibodies. Robust screening procedures have enabled the isolation of antibodies capable of detecting multiple variants of SARS-CoV-2. This versatility underscores their potential application in monitoring viral evolution and the emergence of new variants.

5. DEVELOPMENT OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF VIRUSES OTHER THAN SARS-COV-2

While the importance of monoclonal antibodies in diagnostics became prominently evident during the COVID-19 pandemic, it is noteworthy that even before this global health crisis, monoclonal antibodies were extensively utilized for detecting various viral diseases. Several research groups have reported the development and applications of monoclonal antibodies for detecting viruses other than SARS-CoV-2 [Table 4]. During the selection of targets for antibody development in diagnostic applications, a critical factor is choosing proteins abundant in the virus particle, such as spike and nucleocapsid proteins in the case of SARS-CoV-2, which are also highly immunogenic. These proteins facilitate the production of high-affinity antibodies essential for sensitive detection in immunodiagnostic assays.

Since LFAs can facilitate quick diagnosis and containment efforts, several groups have focused on developing LFA-based tests for detecting viruses other than SARS-CoV-2 as well. Wang et al., developed an LFA-based test for the detection of Varicella-zoster virus (VZV), which causes varicella and herpes zoster [61]. They developed mouse monoclonal antibodies against glycoprotein E present on the surface of VZV in high copy numbers, using hybridoma technology. Upon completing the antibody characterization, the antibodies 2F2 (as capture antibody) and 118H2 (as detector antibody) were selected for LFA. The test allowed the detection of 30 ng/mL VZV gE antigen without any cross-reactivity against Enterovirus 71 or Herpes Simplex Virus 1 and 2. This study emphasizes the potential of monoclonal antibodies for developing specific detection tests with no cross-reactivity to related antigens.

Similarly, Li et. al., developed an LFA-based test using mouse monoclonal antibodies against the envelope (E) protein of the Zika Virus (ZIKV), which is a structural protein present on the surface of the virus [62]. Out of 4 antibodies, antibody 9E-1 was found to be highly specific to ZIKV and had a sub-nanomolar affinity. It was used to develop an LFA-based test along with an antibody B1, and the test was able to detect 33 µg/mL of recombinant E protein and 6.3×10^6 PFU/mL of ZIKV in culture supernatant. Yamaoka et. al., developed an LFA-based test to detect Middle East respiratory syndrome coronavirus (MERS-CoV) [63]. Using hybridoma technology, they developed mouse monoclonal antibodies specific to nucleocapsid protein (122-413 amino acids). Seven antibodies specific to the target protein were tested using immunoprecipitation, which allowed the successful capture of the antigen from cell lysates. Further, sandwich ELISA was used to test all possible combinations of 7 antibodies as capture and detection reagents. Antibody pair 46/20 was found to exhibit maximum reactivity using antigen capture ELISA. It allowed the detection of 0.0625 ng recombinant antigen or 1.5 x 10^5 copies of MERS-CoV virions in a 0.1 mL sample. The same pair allowed the detection of 0.5 ng of purified protein and 3 x 10^6 copies of MERS-CoV virions in a 0.1 mL sample using colloidal gold-based LFA.

Another commonly explored format for developing monoclonal antibody-based diagnostics is sandwich ELISA, which typically offers high sensitivity and quantitative measurement of the target. However, it also necessitates specialized equipment (such as plate readers) and technical proficiency, in contrast to the rapid, user-friendly nature of LFAs, despite their lower sensitivity and limited quantification ability. Several groups have reported the development of sandwich ELISA-based tests to detect viruses. Luo et al., developed a sandwich ELISA-based method for detecting the avian influenza virus (AIV), which can lead to the emergence of zoonotic infections [64]. They developed mouse monoclonal antibodies using hybridoma technology. The specificity of antibody 9F12 was determined using hemagglutination inhibition (HI) against different viruses and was found to be specific to the H3 subtype with no cross-reactivity to other viruses. 9F12 also showed activity in Western blot and IFA and allowed specific detection of H3 strain in clinical specimens using sandwich ELISA. Lee et al. developed a sandwich ELISA to detect MERS-CoV [65]. They developed mouse monoclonal antibodies against the MERS-CoV spike protein. The best antibody pair allowed detection of MERS-CoV S protein with LOD of 5.89 ng/mL using sandwich ELISA. Zai et. al., developed mouse monoclonal antibodies against recombinant glycoprotein of Zaire Ebola virus (rGPdTM; glycoprotein without the transmembrane domain) using hybridoma technology [30]. Two monoclonal antibodies, 6E3 and 3F21, were characterized using multiple assays. In dot blot analysis, 3F21 demonstrated higher sensitivity compared to 6E3, a finding further supported by BLI results indicating that 3F21 exhibited higher affinity. Using these two antibodies in sandwich ELISA allowed the detection of 3.6 ng/mL rGPdTM. Gelanew and Hunsperger produced antibodies against the NS1 protein of the Dengue virus-4 serotype (DENV-4), which is detected by commercially available NS1 antigen tests with limited sensitivity [66]. Three selected antibodies were characterized using Sandwich ELISA, and the best antibody pair, 8A6F2 (capture) and 6D4B10 (detector), exhibited the LOD of 32.5 ng/mL of NS1 protein and allowed specific detection of DENV-4 in cell culture supernatants with no reactivity from other DENV serotypes. Adungo et. al., reported the development of 8 mouse monoclonal antibodies for detecting yellow fever virus (YFV), which spreads through mosquito bites [67]. They employed recombinant envelop protein as well as YF vaccine virus 17D for immunization in mice and successfully isolated 4 antibodies against each target. All the antibodies were found to be highly specific to YFV with no cross-reactivity to related DENV and Japanese Encephalitis virus. All the antibodies also showed reactivity in the IFA. Furthermore, using two antibodies, namely 4C9 (for capture) and 3F4 (for detection), enabled the detection of 1 x 10^3 focus forming units/mL of YFV 17D or 2 ng/well of recombinant envelope protein using sandwich ELISA. This study highlights the utility of monoclonal antibodies for developing cost-effective, highly sensitive, and specific diagnostic tests for arboviruses like YFV that are endemic in regions like Africa, where routine testing measures may be lacking. J. Kim et al., developed mouse monoclonal antibodies against the envelope protein E2 of Chikungunya Virus (CHIKV), which is another virus that spreads through mosquito bites [68]. Four antibodies were characterized using ELISA and Western blot, out of which, two antibodies, 9-1 and 21-1 efficiently recognized CHIKV-E2 protein, and the 9-1 antibody showed no cross-reactivity against other related viruses, such as ZIKV, JEV, and DENV. Further, the antibody 9-1 allowed the detection of as low as 0.7 µg/mL inactivated CHIKV using ELISA and can be explored for the development of new CHIKV diagnostic techniques. Goh et al. worked on a different CHIKV target and developed 11 mouse monoclonal antibodies against the CHIKV capsid protein using hybridoma technology and demonstrated their applications in multiple assays [40]. All antibodies were found to show reactivity against CHIKV using ELISA, Western blot, and immunofluorescence performed using cells expressing recombinant capsid protein or infected with CHIKV. One antibody, 5.5G9, also allowed target protein detection in scattered macrophage-like cells using IHC, demonstrating the versatility of monoclonal antibody-based assays for diagnostics. H. Chen et. al., reported a sandwich ELISA-based method for detecting Tembusu Virus (TMUV), which

causes infection in waterfowls [69]. Using hybridoma technology, they developed 3 mouse monoclonal antibodies against the TMUV envelope protein. The antibodies were characterized using Western Blot and were highly specific to TMUV. Antibody 12B1 was used as a capture antibody with 2D2 for detection to develop TMUV-specific ELISA. The assay was compared to RT-PCR and was found to be 99.1% specific and 93.1% sensitive compared to RT-PCR, further underscoring that monoclonal antibody-based tests are a viable alternative to nucleic-acid-based tests. Sunwoo et al., reported the development of sandwich ELISA for the detection of SARS-CoV, responsible for the first outbreak of SARS in 2002 [38]. They developed mouse monoclonal antibodies using hybridoma technology against the spike protein subunit S1 of the SARS-CoV. Based on the antibody titers, three clones, P135.3F3, P1368D12, and F26G18, were selected for further characterization. They also generated bispecific antibodies by fusion of F26G18 and P136.8D12 hybridoma clones with anti-HRPO hybridoma YP4 to generate quadromas. The use of F26G18 as a coating antibody and its biotinylated version as a detector antibody, sandwich ELISA, allowed the detection of 0.037 µg/mL S1 antigen. Furthermore, the LOD improved to 0.019 µg/mL when they used bi-specific monoclonal antibody F157 (F26G18 x YP4) as the detector antibody, indicating that bispecific antibodies can be explored for enhancing the detection limits of monoclonal antibodies. Competition ELISA has also been explored for the detection of viruses. B. Zhang et al., developed a competitive ELISA for the detection of hepatitis E caused by genotype 1 of the hepatitis E virus (HEV) [70]. They developed 7 mouse monoclonal antibodies against recombinant genotype 1 HEV ORF3 protein using hybridoma technology, out of which 2 antibodies 3C11 and 1D2 were specific to the human HEV SAR-55 strain. Out of the two, one antibody, 1D2 was, showed higher reactivity in competitive ELISA, which can be helpful for large-scale serological testing and clinical diagnosis of HEV infections.

Apart from viruses affecting human health, monoclonal antibody-based tests have been developed to detect viruses responsible for diseases in animals as well. J. Wang et. al., developed four mouse monoclonal antibodies using hybridoma technology against the capsid protein of PCV3, which is a significant cause of disorders such as multi-organ inflammation, nephrotic syndrome, reproductive disorders, and dermatitis in swine [22]. These antibodies were characterized using indirect ELISA, Western Blot, IFA, and Dot Blot, and antibody 7E3 was found to have the highest binding affinity to the target protein. Finally, the B cell epitope of the 7E3 antibody was determined, and an epitope-blocking ELISA (EB-ELISA) was designed to detect PCV3 antibodies in sera, which showed high specificity and sensitivity. This study exemplifies using monoclonal antibodies for monitoring and managing PCV3 infections in swine farms. Similarly, J. Zhang et. al., developed mouse monoclonal antibodies against envelope protein E2 protein using hybridoma technology to detect classical swine fever virus (CSFV), which is a cause of concern in pig breeding industries. After thorough characterization, four antibodies were found to be specific to the sub-genotype 2.1 strain, and out of these, two antibodies, MM1 and MM5, were found to recognize critical epitopes on the E2 protein that were present in 90.9% of the genotype sequences available in GeneBank. Such antibodies can be promising reagents for developing assays like indirect and competition ELISA for detecting CSFV [71]. Guo et. al., also developed monoclonal antibodies using hybridoma technology against the glycoprotein E (gE) of the pseudorabies virus (PRV) that causes porcine pseudorabies (PR) [24]. One monoclonal antibody (1H5) was characterized using indirect ELISA, IFA, and Western Blot and was found to bind to a small epitope conserved in the gE of almost all PRV strains. It can be used to develop antigen detection tests [24]. Skinner et. al., developed mouse monoclonal antibodies against CVV, which is a mosquito-borne virus that causes disease in livestock and humans [23]. Antibodies were developed against inactivated CVV using hybridoma technology, and twelve hybridoma clones were found to show significant reactivity against CVV. Four mAbs, CVV14/15/17, and 18, were found to be highly specific for the detection test of anti-viral antibodies in human sera using IgM-antibody capture ELISA (MAC-ELISA), with MAb CVV14 exhibiting the highest specificity. Antibodies like CVV14 can be used as detector antibodies to develop promising serodiagnostic tools against CVV. Yang et al., developed an ELISA-based method for detecting the Seneca Valley virus (SVV), which has been linked to disease in pigs [41]. Using hybridoma technology, mouse monoclonal antibodies were produced against binary ethylenimine (BEI)-inactivated SVV. Five antibodies were characterized using Dot blot and were found to be specific to SVV. Furthermore, antibody F61SVV-9 exhibited the strongest competition with monospecific polyclonal sera in cELISA and resulted in 100% specificity, indicating that monoclonal antibodies can also be explored for serodiagnosis of viruses using cELISA [41]. The applications of monoclonal antibodies also extend to the accurate and sensitive detection of plant viruses, which is crucial for disease management and control in agriculture. Z. Chen et. al., reported the development of 3 mouse monoclonal antibodies for the detection of Zucchini yellow mosaic virus (ZYMV) using hybridoma technology [72]. Using ZYMV virion for immunization, three hybridoma clones, 16A11, 5A7, and 3B8, were developed and characterized using multiple immunoassays.

6. CHALLENGES AND FUTURE PERSPECTIVE

Monoclonal antibodies have revolutionized viral diagnostics by offering unparalleled specificity and sensitivity in detecting viral pathogens. Hybridoma technology and phage display have been instrumental in producing antibodies with high affinity and tailored cross-reactivity against a wide range of viral targets. These antibodies are crucial components in various immunodiagnostic formats, including ELISAs, LFAs, Western blots, and IHC, providing adaptable solutions for different diagnostic needs. The COVID-19 pandemic underscored their pivotal role in rapid and accurate diagnostics, particularly through LFAs for large-scale screening efforts. Beyond SARS-CoV-2, monoclonal antibodies continue to contribute significantly to detecting diverse viral diseases in human, animal, and plant hosts. The ability to generate monoclonal antibodies that can detect multiple viral strains or variants further augments their utility in monitoring the emergence of new threats.

However, several challenges must be addressed to tap into the full potential of monoclonal antibody-based diagnostic methods. The overall antibody discovery and production process can be expensive, limiting its use in resource-constrained settings. Additionally, viruses continuously evolve, necessitating frequent updates to the antibodies to maintain the diagnostic potential of the tests. Intensive screening is required to select antibodies that do not cross-react with antigens other than the target.

These limitations are actively being addressed by scientists worldwide. Expression hosts and strategies to improve antibody yields are being explored [73]. Innovations are also underway in the area of biosensors to

improve the detection sensitivity of assays [74]. Integration of artificial intelligence into the antibody discovery and engineering pipelines is expected to reduce the overall production timeline and will make it easier to keep up with the emerging viral pathogens [59]. Additionally, while this review focuses on the diagnostic applications of monoclonal antibodies, it is important to note their potential as therapeutic agents and research tools, which expand their impact in combating viral infections and understanding viral biology. Monoclonal antibodies have shown promise as potential therapeutic agents for viral diseases, either by directly neutralizing the virus or modulating the host immune response [75]. Additionally, their use as research tools in immunoprecipitation, immunofluorescence, and IHC has provided valuable insights into viral protein functions and cellular localization [76].

7. CONCLUSION

In conclusion, the development of monoclonal antibodies has revolutionized the field of viral diagnostics, offering the desired specificity and sensitivity in detecting viral pathogens. As the threat of emerging and re-emerging viral diseases continues to challenge global health, the importance of robust diagnostic tools cannot be overstated. With their versatility and potential for further engineering, monoclonal antibodies will undoubtedly remain at the forefront of efforts to combat viral diseases through early and accurate detection, paving the way for timely interventions and improved clinical outcomes.

8. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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10. CONFLICTS OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

11. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

12. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

14. PUBLISHER'S NOTE

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