JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S9

# A Review of Paper-Based Diagnostic Chips: Redefining Laboratory Viral Detection

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## ABSTRACT

Viruses have posed significant threats to human health throughout history, causing substantial disruptions and millions of deaths. Traditional viral detection methods, such as electron microscopy, cell culture, immunofluorescence assays, and reverse transcription polymerase chain reaction (RT-PCR), have limitations in terms of cost, time, and the need for skilled personnel. Paper-based diagnostic chips have emerged as a promising alternative, offering advantages such as affordability, portability, and user-friendliness. These devices are constructed from porous membranes coated with capture molecules that selectively bind to target pathogens. Recent advances in biosensor research have focused on developing faster, more affordable, and highly reliable diagnostic devices for viral detection. Paper-based diagnostic chips have demonstrated superior sensitivity, specificity, and selectivity compared to traditional methods in clinical contexts. Recent studies have yielded promising results for detecting various viruses, including hepatitis B, influenza, human papillomavirus, Ebola, herpes, HIV, hepatitis C, Zika, respiratory syncytial virus, and SARS-CoV-2. However, challenges such as sensitivity, selectivity, and signal stability must be addressed to achieve consistent and accurate pathogen detection. Despite these limitations, paper-based diagnostic chips represent a promising frontier in viral diagnostics, with ongoing research and technological advancements expected to deliver even more efficient, sensitive, and robust solutions in the future.

**Keywords**: virus, viral detection, laboratory diagnosis

## Introduction

Viruses have posed significant threats to civilization for many years, causing substantial disruptions, from the outbreaks of yellow fever to the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. The existence of viruses was first acknowledged with the discovery of the tobacco mosaic virus in 1892 (Bos, 1999), followed by the foot-and-mouth disease virus in 1898 (Madin, 2011, p. 2). In 1901, the yellow fever virus was identified as the first virus known to infect humans. Currently, approximately 200 viruses are known to infect humans (Woolhouse et al., 2012), with most being zoonotic in origin. Zoonotic viruses are those that originate in animals and are transmitted to humans (Murphy, 2008). Direct transmission of these viruses can occur through contact with an infected host (Brauburger et al., 2012), vaginal or oral intercourse (Louten, 2022), animal bites (Weyer et al., 2011, pp. 1983–200), or neonatal transmission (Louten, 2022). Indirect transmission pathways include food (Wang et al., 2017), fomites (Ganime et al., 2016), direct contact with human fluids (Shears & O'Dempsey, 2015), and vectors. Additionally, viruses can spread through aerosols or droplets in certain cases (Seto, 2015). Once inside the human body, viruses can invade through various mechanisms and lead to a broad spectrum of symptoms and diseases, ranging from mild to severe and sometimes fatal outcomes (Van Vliet et al., 2017).

Historically, numerous pandemics and epidemics have been recorded, causing millions of deaths and the collapse of civilizations and economies (Uddin & Acter, 2021). The World Health Organization (WHO) continuously monitors and

documents disease outbreaks in an online database. Viral diseases in humans are highly contagious and often exhibit high fatality rates. Therefore, early detection and accurate diagnosis are critical for controlling and preventing epidemics or pandemics. There is an urgent need for the development of rapid, sensitive, and cost-effective technologies for detecting viruses and diagnosing associated infections. Biosensors represent one of the most promising tools in this regard. These devices are analytical instruments that incorporate a biological sensing component (Viswanath et al., 2018). By combining the sensitivity and specificity of biological systems with physicochemical transducers, biosensors enable advanced bioanalytical measurements in simple and user-friendly formats (Ozer & Henry, 2021).

Biosensors offer several advantages, including affordability, real-time detection, user-friendliness, minimal sample preparation, simplicity, and portability. In contrast, traditional analytical methods are generally costly, time-consuming, require skilled professionals, involve more extensive sample preparation processes, and depend on high-tech equipment. Consequently, biosensors are increasingly preferred for identifying viral infections. Among biosensors, paper-based sensors have garnered significant interest due to their simplicity, biocompatibility, cost-effectiveness, biodegradability, and ability to facilitate fluid movement through capillary action. This review examines traditional methods for detecting human viruses, focusing on their mechanisms, benefits, and limitations. Additionally, the review explores the rapidly emerging paper-based detection methods, which have gained popularity for their advantages. It also discusses the efficiency, specificity, and speed of detection of biosensor technologies, as well as the limitations and potential of paper-based diagnostic chips for detecting human viruses.

**Existing Viral Diagnostic Methods and Their Limitations**

Traditional viral detection methods are widely used in clinical settings across various regions, albeit with differing costs, quality, and availability. These approaches can be broadly categorized into direct and indirect methods (Burrell et al., 2017). Direct methods involve the detection of viral particles, antigens, or nucleic acids, while indirect methods examine the effects of the virus, such as cytopathic effects or the presence of antibodies.

Electron microscopy is a direct method that enables rapid and accurate detection of viruses by allowing direct visualization of samples without the need for molecular probes (Richert-Pöggeler et al., 2019). This technique offers high precision and resolution, facilitating quick identification of viruses. However, the equipment required for electron microscopy is expensive and requires regular maintenance to ensure optimal performance. Moreover, skilled and experienced technicians are necessary for accurate identification of viruses (Zhang et al., 2013).

Cell culture, a commonly used indirect detection method, is particularly prevalent in developing countries due to its lower cost compared to other techniques. However, this approach requires samples to be incubated for days or even weeks before observing cytopathic effects (CPE), which are used to identify the virus. The extended incubation period also makes this technique prone to contamination. Additionally, cell culture can only provide presumptive identification of viruses based on the type of cell culture, incubation conditions, incubation duration, and observed

CPE. Confirmation tests, such as immunofluorescence (IF) assays, are often necessary for definitive identification (Hematian et al., 2016).

Immunofluorescence assays utilize antibodies conjugated with fluorophores to bind specifically to targeted antigens for virus detection. However, this technique is susceptible to cross-reactivity and contamination, particularly when detecting viruses within the rhinovirus family. Moreover, certain viruses, such as specific serotypes within the enterovirus family, cannot be detected using IF assays (Joshi & Yu, 2017).

The shell vial technique, a modified indirect method, employs small vials containing a specific monolayer of growing cells (Jayakeerthi et al., 2006). In this method, a patient's specimen is transferred into a smaller vial and centrifuged, significantly reducing the incubation period to a few hours or days. Viral detection is then performed using fluorescent antibodies. This approach has demonstrated greater speed and efficiency in virus isolation and has been adapted for various new cell culture formats, including cryopreserved cell cultures. These advancements have simplified cell preservation and maintenance when stored in shell vials. Additionally, the shell vial technique allows for viral isolation using cultured cells, wherein different cell types are grown as monolayers within a single vial to detect multiple viruses (Caceda & Kochel, 2007).

Despite the improved accuracy and sensitivity of the shell vial technique compared to traditional cell culture methods, limitations persist. The availability and type of cell lines used for a given virus can significantly impact the accuracy of isolation and identification. As a result, this technique is limited to detecting only certain viruses (Hematian et al., 2016).

Immunoassays represent another category of indirect detection techniques commonly employed in clinical practice for virus identification. Various immunoassay methods, including enzyme-linked immunosorbent assays (ELISA), lateral flow immunoassays (LFIA), western blot (WB), and agglutination assays, are used to detect antibodies produced by the immune system in response to viral infections (Ahsan, 2022). These methods are highly detailed, sensitive, and precise. However, their accuracy heavily depends on the quality of the reagents and procedures used, and the required equipment and chemicals are expensive. Additionally, many immunoassay protocols are labor-intensive (Souf, 2016).

For instance, a study comparing IgG ELISA to hemagglutination inhibition (HI), another indirect assay used to distinguish between primary and secondary dengue infections, demonstrated the superiority of IgG ELISA in differentiating these infection types. While both tests were time-intensive, the IgG ELISA kit, which was commercially available, offered greater ease of use and quicker results compared to the HI test. Moreover, IgG ELISA kits were more readily accessible in regions like Indonesia, where dengue fever is prevalent (Lukman et al., 2016).

Reverse transcription polymerase chain reaction (RT-PCR) is widely regarded as a standard diagnostic method for detecting viral infections. However, this

technique is not without limitations, as it can yield false-positive or false-negative results (Tahamtan & Ardebili, 2020). Although RT-PCR is extensively employed, the global demand for rapid, affordable, reliable, and easily accessible molecular diagnostic tools far exceeds its testing capacity and availability. Challenges associated with RT-PCR include specimen collection and handling, amplification and detection of viral RNA, and ensuring clinical sensitivity and specificity. In addition to RT-PCR, other molecular detection methods, including ELISA, serum neutralization assays, clustered regularly interspaced short palindromic repeats (CRISPR), and next-generation sequencing (NGS), have been utilized. Nevertheless, these techniques also present limitations, being expensive, time-consuming, and requiring skilled personnel for operation (Jasim, 2021; Kim et al., 2018; Meligy et al., 2016).

**Composition and Types of Pathogen-Sensing Paper**

Paper-based diagnostic chips are generally constructed from porous membranes made of cellulose or nitrocellulose, coated with capture molecules like antibodies or aptamers. When a sample is applied to the membrane, the capture molecules selectively bind to the target pathogen, enabling its detection. Paper, a three-dimensional material formed by hydrogen bonding between the hydroxyl groups of cellulose fibers, is produced by pulping, combining, fabricating, and processing plant-based raw materials. During pulping, various fillers, including synthetic additives and colorants, can be incorporated to impart specific properties. Processes such as sizing, calendaring, and drying help shape the paper and reduce water absorption, increasing its strength (Khan et al., 2020; Sjöström & Alén, 1998).

Further processing, such as the addition of pigment coatings and calendaring, can create a smoother surface and reduce pore size. Mineral fillers like calcium carbonate, clay, and starch are often included to improve light scattering, ink absorption, and smoothness. Paper characteristics can also be modified by altering the length, diameter, and chemical properties of cellulose fibers. Long fibers contribute to strength, while shorter fibers fill gaps to reduce pore size and enhance durability (Lin et al., 2016; Zhang et al., 2018).

Filter paper, made exclusively from natural cellulose impurities, is the most used substrate in paper-based microfluidic sensors (Kung et al., 2019). For example, Whatman No. 1 paper, with its uniform texture, moderate flow rate, and thickness of 0.18 mm, is often used to develop these sensors. However, when standard filter paper lacks the necessary properties, alternative types of paper or modifications to its structure are explored. Cellulose-based paper's unique microstructure allows the creation of innovative devices with distinctive properties (Yun et al., 2023).

Nitrocellulose paper is particularly notable for its efficient protein-binding capabilities, making it widely used in the paper-based sensor industry. Lu et al. demonstrated the development of paper-based sensors using nitrocellulose films as substrates, with colorimetric analysis facilitated by enzymes following wax barrier creation through printing and heating (Lu et al., 2010; Tang et al., 2022). Jiang et al. developed microfluidic detection devices using nitrocellulose films, glass cellulose films, and filter paper to detect bladder cancer. By combining nitrocellulose's protein immobilization capability with the superior water absorption properties of filter paper, they ensured dynamic sample flow (Jiang et al., 2020).

Translucent paper, with enhanced mechanical properties and excellent optical transparency compared to conventional paper, serves as a promising substrate for novel paper-based technologies (J. Jin et al., 2016; H. Zhu et al., 2014). Zong et al. developed a transparent paper-based chemical sensing system capable of detecting multiple targets with high sensitivity and low cost. This system successfully detected blood cholesterol and bovine serum albumin with detection limits of 0.1 M and 0.1 mM, respectively (Zong et al., 2019). Ying et al. created a pump-free, paper-based microfluidic analytical device using nanofibrillated cellulose (NFC) paper for glucose detection, achieving a detection limit of 1.4 mM. The authors also demonstrated surface-enhanced Raman scattering-based photochemical detection enabled by the high transparency of NFC paper-based devices (Ying et al., 2020).

**Recent Trends in Biosensor Research for Rapid and Accurate Viral Detection**

Given the global impact of viruses, particularly in resource-limited settings, biosensor research has increasingly emphasized the development of faster, more affordable, and highly reliable diagnostic devices. For instance, Adnane et al. developed a biosensor capable of detecting the rabies virus and H7N1 antigen by immobilizing antibodies on gold-thiol electrodes and measuring impedance. This device demonstrated detection limits of 0.5 and 5 µg/ml for rabies and H7N1 antigens, respectively, by evaluating the affinity interaction between the antibodies and their specific antigens (Adnane, 2011).

Chuang et al. developed a highly sensitive and rapid biosensor for detecting the hepatitis B virus (HBV) using a cost-effective, disposable polycarbonate-based surface plasmon resonance (SPR) sensing cartridge (Chuang et al., 2012). This device is portable, requires minimal sample volume, and enables on-site detection with a quick turnaround time of 3 minutes for concentrations between 20000 and 2 pg/mL, and up to 17 minutes for the lowest limit of detection (2 fg/mL). Similarly, an electrochemical DNA biosensor using a nanoporous gold platform was employed for HBV detection (Ahangar & Mehrgardi, 2017). This biosensor utilized the covalently attached ferrocene as a redox reporter on the DNA probe to generate an electrochemical signal. By integrating a nanoporous gold electrode, the sensitivity was enhanced, enabling detection of the HBV genome in real samples with fewer PCR cycles. This method allowed the distinction between healthy individuals and HBV patients, producing mutant DNA with a linear dynamic range of 0.4 to 10 nmol and a reliable reproducibility (RSD) of 8.9%.

Nidzworski et al. developed a universal immunosensor capable of detecting all influenza A virus serotypes (Nidzworski et al., 2014). Using electrochemical impedance spectroscopy (EIS) and direct immobilization, the device was miniaturized to the size of a pen or a glucose-monitoring strip. The system, which includes a central reader unit and interchangeable components for individual patient use, significantly reduced production costs while maintaining high mobility, making it suitable for practical applications. Its adaptability allows for diagnostic usage in both human and veterinary contexts. The method's high sensitivity enables early detection in birds

during the initial infection stages, facilitating prompt epidemic management and sanitary measures. A detection limit of 20 pg/mL, corresponding to a greater than 5% change in electron transfer resistance, was competitive with other existing techniques.

In another study, a low-cost, portable graphene-enabled biosensor with a monoclonal antibody specific to Zika virus (ZIKV) antigens was developed for the early detection of ZIKV. Afsahi et al. designed a biosensor that could selectively differentiate the NS1 antigen of the Japanese encephalitis virus (JEV) from ZIKV. Using the Agile R100 biosensor chip, the device detected ZIKV NS1 at concentrations as low as 0.45 nM (Afsahi et al., 2018). Prabowo et al. created a portable SPR biosensor to detect and quantify human enterovirus 71 (EV71) . This device employed an SPR technique offering real-time detection, high sensitivity, and label-free assays with fluorescence, suitable for outbreaks. Detection limits for EV71 were significantly lower, at 67 vp/mL and 4.8 pg/mL, when compared to conventional methods such as ELISA and viral plaque assay (VPA) by utilizing the VP1 biomarker in a phosphate buffer solution.

Jin et al. devised a surface-based impedimetric biosensor for label-free dengue virus detection with a limit of detection (LOD) of 1 fM, employing functionalized graphene oxide (GO)-wrapped SiO2 particles (S.-A. Jin et al., 2016). Kanagavalli et al. used an electrochemically active ruthenium bipyridine complex bonded to the surface of GO for dengue virus detection, achieving LODs of 0.38 ng/mL and 0.48 ng/mL, respectively, in chronoamperometric and fluorescence quenching-based immunoassays (Kanagavalli & Veerapandian, 2020). Omar et al. developed an optical biosensor utilizing the SPR of CdS quantum dots composited with amine-functionalized GO thin films for dengue virus detection (Omar et al., 2020). This biosensor demonstrated a detection limit of 0.001 nM/1 pM with a sensitivity of 5.49° $nM^{-1}$ for detecting DENV E-protein. A related approach employed an rGO-polyamidoamine nanocomposite, which improved performance, yielding an LOD of 0.08 pM (Omar, Fen, Abdullah, Mustapha Kamil, et al., 2020).

Lee et al. enhanced the sensitivity of a GO-based biosensor for dengue detection using a fluorometric method combined with loop-mediated isothermal amplification, achieving an LOD of 2.1 nM (Lee et al., 2020). Kamil et al. developed a GO-integrated biofunctionalized tapered optical fiber-based sensor with an LOD of 1 pM and a sensitivity of 12.77 nm/nM (Kamil & Are, 2018). Navakul et al. employed electrochemical impedance spectroscopy (EIS) with a GO-reinforced polymer to detect the dengue virus, achieving an LOD of 0.12 plaque-forming units (PFU)/mL (Navakul et al., 2017).

Zeng et al. developed immunochromatographic strips using gold nanoparticle (GNP)-tagged antibodies targeting the S protein of SARS-CoV-2. These strips facilitated rapid, qualitative detection of IgG and IgM antibodies against SARS-CoV-2 in patient blood samples within 15 minutes (Zeng et al., 2020). The assay demonstrated high sensitivity and specificity, making it suitable for screening both symptomatic and asymptomatic carriers of COVID-19.

## The Role of Paper-Based Diagnostic Chips in human Viral Detection

Paper-based diagnostic chips hold immense promise for point-of-care (POC) testing in the medical field. Replacing traditional diagnostic procedures, which often require entire laboratory setups, with compact, coin-sized diagnostic tools could significantly improve healthcare access in resource-limited regions. In industrialized countries, citizens benefit from comprehensive health examinations and treatments, while millions in low-income nations die annually due to undiagnosed, untreated viral infections (Silasi et al., 2015; Stillwaggon, 2002). Paper-based chips address these disparities by offering cost-effective, portable solutions that eliminate the need for bulky laboratory equipment.

Advantages of paper-based diagnostics include disposability, minimal sample and reagent volume requirements, and rapid turnaround times for detection (Teengam et al., 2017; X. Zhu et al., 2018). Despite their compact size, these devices have shown superior sensitivity, specificity, and selectivity compared to traditional methods in clinical contexts. Owing to its affordability, self-powered fluidics, and widespread availability, paper has become the preferred substrate for POC devices (Mahato et al., 2017).

The overarching goal of paper-based diagnostics is to provide equitable diagnostic tools for individuals across all socioeconomic strata. This innovation significantly impacts POC diagnostics in both fieldwork and personalized healthcare. As emphasized in a 2013 policy paper by the Infectious Disease Society of America (IDSA) (Caliendo et al., 2013), effective diagnostic tools must deliver results within an hour, maintain high accuracy, exhibit sensitivity, and be easy to use. Paper-based diagnostic chips fulfill these criteria, advancing the accessibility and effectiveness of viral detection.

## Recent Advances in the Development of Paper-Based Diagnostic Chips for Human Virus Detection

Fabrication, application, and integration are essential aspects of paper-based diagnostic tools that have been extensively studied. Recent investigations, such as those by Chen et al., have yielded promising results utilizing the electrochemiluminescence (ECL) method for detecting hepatitis B virus surface antigens. This approach involved attaching a 6 mm-diameter paper disc to a screen-printed electrode. Samples were applied to the paper, and subsequent activity detection was performed using cyclic voltammetry and ECL, facilitated by a custom-built ECL detector. The device successfully detected the virus at a concentration of 34.2 pg/mL (Chen et al., 2018). Despite its low cost and ease of use, limitations such as a restricted linear response range and an inability to achieve lower detection limits compared to other immunoassays remain issues that require further investigation (Chen et al., 2014; Shourian et al., 2015). Nevertheless, this method demonstrated high reliability, stability, reproducibility, and selectivity when compared to chemiluminescent immunoassays and ELISAs for hepatitis B virus detection.

Another paper-based electrochemical immunosensor for the label-free detection of influenza virus H1N1 was developed by (Devarakonda et al., 2017). Single-walled carbon nanotubes functionalized the carbon electrodes, and sensitivity was enhanced by incorporating chitosan. Hydrophobic silica nanoparticles were sprayed onto the paper's surface 30–40 times, while antibodies specific to the target analytes were immobilized using glutaraldehyde cross-linking. This device detected the virus within 30 minutes at concentrations as low as 113 PFU/mL. The study aimed to produce a portable, cost-effective, and disposable device for detecting viruses and bacteria, particularly in resource-limited settings.

Grant et al. developed a paper-based lateral flow device to monitor the status of human papillomavirus (HPV) vaccine recipients (Grant et al., 2016). Using nitrocellulose as a substrate, type 16 HPV particles were immobilized to differentiate between high and low levels of type 16 HPV antibodies in plasma. This device was tested on 35 individuals, of whom 28 were eligible for testing. These individuals included ten who had not received the HPV vaccine, three who had received one dose, five who had received two doses, and ten who had received three doses (Grant et al., 2016). The single-blind study results successfully differentiated immunized participants from those who were not, although one test from an unvaccinated individual was interpreted differently by the researchers. Despite this discrepancy, the method demonstrated potential for customization in various screening and serological assays.

Lei et al. modified traditional ELISA to create a colorimetric, paper-based sandwich immunoassay for detecting influenza viruses (Lei et al., 2015). Using purified nucleoprotein (NP) protein and influenza A H1N1 virus as models, the system utilized only 5 μL of sample and reagent. Gold enhancement solutions were used to generate additional gold nanoparticles (AuNPs) conjugated with secondary antibodies in an enzyme-free sandwich model. This approach achieved detection limits of $2.6 \times 10^3$ PFU/assay for H1N1 and $2.7 \times 10^4$ PFU/assay for H3N2, offering cost-effective, rapid, and highly sensitive detection methods (Lei et al., 2015).

Bhardwaj et al. combined electrochemical and colorimetric techniques to detect the influenza H1N1 virus using a dual-layer paper sample pad (Bhardwaj et al., 2019). The system concentrated horseradish peroxidase-tagged antibody-H1N1 complexes to detect low viral loads, achieving a detection limit of 4.7 PFU/mL by electrochemical impedance spectroscopy (EIS) and 2.27 PFU/mL by colorimetry within a six-minute turnaround time. This dual-modality system reduced the likelihood of false positives and demonstrated potential for sensitive detection.

In addition to antibody-antigen detection, nucleic acid-based detection methods are being developed for paper-based platforms. Magro et al. created a paper-based microfluidic device utilizing isothermal reverse transcription and recombinase polymerase amplification (RT-RPA) to identify Ebola virus RNA. By freeze-drying the RT-RPA solution onto paper, samples were tested by heating to approximately 40 °C, with fluorescence changes indicating results (Magro et al., 2018). A detection limit of $10^7$ copies/μL was achieved in 20 minutes, although this was higher than tube-based tests. Teengam et al. designed a paper-based electrochemical biosensor employing inkjet-printed graphene polyaniline and peptide nucleic acids (PNA) probes for

detecting HPV DNA, achieving a detection limit of 2.3 nM. This device offered a low-cost, disposable platform with potential applications in early cancer screening (Teengam et al., 2017).

Narang et al. incorporated zinc-silver nanoblooms into a paper-based electrochemical sensor to detect herpes virus 5 (HHV-5) DNA. This device achieved a detection limit of 97 copies/mL within 5 seconds, representing a significant advancement in diagnostic capabilities for clinical and point-of-care applications (Narang et al., 2018).

Tang et al. enhanced lateral flow assay (LFA)-based HIV detection by applying a dialysis concentration approach. This method improved detection sensitivity tenfold, achieving a detection limit of 0.1 nM within 25 minutes. Replacing traditional ultrafiltration methods with paper-based technology streamlined the concentration process while maintaining accuracy (Tang et al., 2016).

Teengam et al. proposed a paper-based DNA sensing platform for fluorescence detection of hepatitis C virus (HCV) DNA. This device achieved a detection limit of 5 pmol/spot using a PNA probe and ssDNA-specific fluorescent dye, enabling label-free imaging of DNA targets (Teengam et al., 2017). Similarly, Sabalza et al. repurposed a microfluidic HIV RNA detection device for Zika virus RNA in saliva. By combining RT-LAMP with reverse dot blot (RDB), the system achieved a detection limit of $8.57 \times 10^2$ viral copies/mL within 3–10 minutes, providing a rapid and adaptable platform for emerging viral infections (Sabalza et al., 2018).

Cao et al. illustrated the use of a paper-based cell-free system incorporating toehold switch sensors and NASBA for the specific and sensitive detection of respiratory syncytial virus (RSV) subtypes A and B. After screening synthetic RNAs from closely related respiratory viruses, the SB2 and SA13 toehold-switch sensors exhibited excellent specificity (Cao et al., 2021). When paired with NASBA, detection limits were determined to be 91 aM for RSVB and 5.2 fM for RSVA. This method demonstrates the potential for broad applicability by modifying and reconstructing toehold-switch sensors for various target species.

Wen et al. developed a point-of-care (POC) serological assay for detecting SARS-CoV-2 IgG antibodies. This technique employed a lateral flow immunoassay (LFIA) where the SARS-CoV-2 nucleocapsid protein was immobilized on the strip's surface, and anti-human IgG was conjugated with colloidal AuNPs (Wen et al., 2020). Parameters such as antigen concentration, bovine serum albumin blocking concentration, and conjugation pH were optimized. Using 80 μL of analyte solution (10 μL of serum and 70 μL of sample diluent), the assay provided results in 15–20 minutes. This method offers a rapid preliminary test for clinicians, enabling timely diagnosis and effective treatment initiation. Furthermore, the LFIA technique has proved invaluable for serological monitoring of large populations, particularly in determining early exposure to SARS-CoV-2 in epidemiological studies. Compared to conventional isolation and ELISA methods, LFIAs are faster, more accessible, and

cost-effective, making them suitable for public health efforts, especially in resource-constrained areas.

Linnes et al. applied the microRapid Autonomous Analytical Device for detecting SARS-CoV-2 RNA. This RT-LAMP-based platform could identify as few as 75 RNA copies in saliva samples within 30 minutes. To further reduce turnaround times and increase testing efficiency, Azzi et al. introduced a rapid salivary test (RST) based on LFIAs for SARS-CoV-2 detection. This antibody-based assay identified spike proteins in saliva in under 10 minutes (Azzi et al., 2020). These advancements in paper-based diagnostics suggest a future with affordable, portable, and user-friendly tools. Developing countries stand to gain the most from such technologies, which address challenges related to disposal, handling, and portability.

**Challenges and Future Directions**

Paper-based diagnostic chips exhibit significant potential in detecting and monitoring viral infections. These platforms offer advantages such as portability, affordability, disposability, quick processing times, and user-friendliness. However, critical issues like sensitivity and selectivity must be resolved to achieve consistent and accurate pathogen detection. Researchers are continually working to enhance these platforms, recognizing paper-based diagnostics as leading contenders in viral detection technology.

One challenge with paper-based devices is the diminished signal intensity when detection solutions remain on the device for extended periods before use (Magro et al., 2018). Like other conventional technologies, the quality of paper-based diagnostics must be maintained even as devices are downsized. It is essential to optimize the designs to ensure outcomes remain on par with gold-standard methods. Additionally, paper-based devices face unique challenges linked to the processes they employ. For instance, the interpretation of data from colorimetric devices can vary due to factors like light intensity and wavelength during readings. Data acquired from digital cameras or scanners are also susceptible to variability depending on ambient conditions and equipment specifications.

Electrochemical-based devices often require expensive laboratory tools for accurate result interpretation due to issues such as deactivation of immobilized recognition elements, including antibodies or enzymes. Chemiluminescence-based systems face limitations due to the transient nature of signals, which fade over time. Electrochemiluminescence devices, while generating longer-lasting signals, produce lower intensities and are restricted to detecting small molecules containing tertiary amine groups due to the ruthenium complex utilized in the assay.

The primary goal of paper-based diagnostics is to provide reliable, portable tools for point-of-care or field-based testing. Current research focuses on integrating and improving traditional diagnostic methods, adapting them for miniaturized paper-based formats. The use of paper significantly reduces production costs, assay size, sample volume requirements, and processing times. Furthermore, multiplex detection on paper platforms allows simultaneous analysis of multiple analytes without significantly increasing manufacturing expenses.

Overall, paper-based diagnostic chips represent a promising frontier in viral diagnostics. Ongoing research and technological advancements are anticipated to deliver even more efficient, sensitive, and robust diagnostic solutions in the future.

**Conclusion**

Paper-based diagnostic chips are emerging as a transformative innovation in the field of viral diagnostics, offering the potential to address the limitations of conventional detection methods. These devices provide portability, affordability, disposability, rapid detection, and ease of use, making them especially beneficial for point-of-care applications in resource-limited settings. Recent advancements have demonstrated significant progress in improving the sensitivity, specificity, and reliability of these tools for a variety of viruses, including respiratory syncytial virus, SARS-CoV-2, and human papillomavirus.

However, challenges such as signal stability, quality consistency, and detection limits remain critical areas for improvement. Addressing these limitations requires continuous innovation in fabrication techniques, optimization of detection elements, and integration of advanced technologies to enhance the overall performance of these devices. As research in this field progresses, paper-based diagnostic chips are expected to revolutionize viral detection and monitoring, contributing significantly to global health, particularly in underserved regions. Their ability to miniaturize traditional laboratory procedures into compact, cost-effective formats represents a critical step toward achieving universal access to reliable diagnostics.

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