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Invited Review

Current trends and future potential in the detection of avian coronaviruses: An emphasis on sensors-based technologies

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ABSTRACT

Infectious bronchitis virus (IBV), an avian coronavirus, member of the genus *Gammacoronavirus*, poses significant threats to poultry health, causing severe respiratory, reproductive, and renal infections. The genetic diversity of IBV, driven by mutations, recombination and deletions, has led to the emergence of numerous serotypes and genotypes, complicating both diagnosis and control measures. Rapid and accurate diagnostic tools are essential for effective disease management and minimizing economic losses. Conventional diagnostic methods, such as PCR, virus isolation, and serological assays, are hindered by limitations in sensitivity, specificity, and turnaround time. In contrast, innovative biosensor platforms employing advanced detection mechanisms-including electrochemical, optical, and piezoelectric sensors-offer a transformative solution. These technologies provide portable, highly sensitive, and rapid diagnostic platforms for IBV detection. Beyond addressing the challenges of conventional methods, these biosensor-based approaches facilitate real-time monitoring and enhance disease surveillance. This review highlights the transformative potential of biosensors and their integration into diagnostic strategies for avian coronavirus infections, presenting them as a promising alternative for precise and efficient IBV detection.

1. Introduction

Infectious bronchitis virus (IBV), classified within the gamma or Group-3 coronaviruses of the order *Nidovirales* and family *Coronaviridae*, is characterized by a positive-sense, single-stranded RNA genome. The genome is approximately 27 kb in length and organized as follows: 5′UTR-1a/1 ab-S-3a-3b-E-M-5a-5b-N-3′UTR (Legnardi et al., 2020). The virions of IBV are enclosed in a spike-like projection, which is approximately 20 nm in size (Cavanagh and Gelb, 2008). Morphologically, coronaviruses are classified into four groups based on antigenic cross-reactivity and nucleotide sequence similarities (Papineau et al., 2019). Alpha or Group-1 coronaviruses include human coronavirus, porcine transmissible gastroenteritis coronavirus (TGEV), and porcine respiratory coronavirus (PRCV). Beta or group-2 coronaviruses include severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 (COVID-19), middle-east respiratory syndrome coronavirus (MERS-CoV), mouse hepatitis coronavirus (MHV), and bovine coronavirus (BCoV) (Decaro and Lorusso, 2020; Khan et al., 2023), whereas gamma or group-3 and group-4 coronaviruses usually infect all types of poultry, especially chickens, pheasants, and galliforms (Haake et al., 2020).

IBV is a highly contagious viral disease that poses a significant economic problem in commercial chicken farms. Initially manifesting as a respiratory disease, IBV can also affect the urogenital, oviduct, and gastrointestinal tracts, depending on strain variation and tissue tropism (Ramakrishnan and Kappala, 2019; Ennaji et al., 2020). The disease can lead to high mortality rates in broiler or breeder chickens due to secondary infections. Notably, the disease can spread in both vaccinated

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and non-vaccinated poultry farms (Khataby et al., 2016; Bhuiyan et al., 2024). Cross-protection between different variants and live vaccines has led to outbreaks in vaccinated flocks. IBV outbreaks are common due to the emergence of strains with high mutation rates and replication in the hypervariable spike (S) gene region. Despite the use of inactivated and live attenuated vaccines, outbreaks remain persistent. Complete eradication of IBV infection is challenging due to the lack of cross-protection against diverse genotypes, variants, and serotypes. Identifying specific potential IBV genotypes or strains in different geographic regions is crucial for developing new vaccination strategies against the disease (Lin and Chen, 2017; Gallardo, 2021).

The limitations of traditional diagnostic techniques, including extended processing times and dependence on specialized equipment and knowledge, have led to a transition in diagnostic practices with the emergence of biosensor technology. In recent years, biosensor technology has revolutionized the field of diagnostics, offering rapid, sensitive, and portable platforms for pathogen detection (Bhatia et al., 2024). These novel biosensors hold immense promise for enhancing the diagnosis of avian coronavirus infections in poultry. Additionally, biosensors can offer sensitivity and specificity comparable to or even surpassing that of molecular techniques, making them reliable alternatives for virus detection. With advancements in biosensor technology, there is potential for further improvements in sensitivity and specificity, enhancing their utility in virus surveillance and diagnosis.

Although biosensor technology offers numerous advantages, including portability, high sensitivity and rapid pathogen detection, several challenges must be addressed for widespread implementation. On of the key challenges is the cost and economic feasibility, as biosensors—particularly those utilizing advanced materials like nanomaterials, can be expensive to develop and manufacture, potentially limiting their accessibility in resource-limited settings or for smallholder farmers. Scalability is another significant concern, as the transition from laboratory prototypes to mass production poses technical and logistical challenges. Variability in manufacturing processes and quality control can further impact device performance, underscoring the need for stringent standardization. Additionally, the lack of standardization in biosensor development and performance evaluation complicates their comparison with traditional diagnostic methods and hampers validation efforts. Moreover, regulatory approval processes for biosensor technologies can also be time-intensive, further delaying their market availability. Addressing these challenges is critical to unlocking the full potential of biosensors for pathogen detection and ensuring their adoption across diverse settings.

This review explores current and future diagnostic approaches for IBV, with a particular emphasis on biosensor-based diagnostic tools. We discuss potential methods to boost sensor performance by utilizing new materials such as functionalized surfaces, biomimetic receptors, and nanomaterials. Additionally, we highlight the challenges in transitioning biosensor technology from the laboratory to field operations, including issues related to standardization, validation, affordability, and regulatory approval.

2. Clinical features of IBV infection

IBV is a prevalent respiratory infection in birds, causing symptoms like tracheal or respiratory rales, gasping, sneezing, coughing, head-shaking, and nasal and ocular discharge (Abdelaziz et al., 2019). Severe cases can lead to dyspnea and lethargy, with some strains causing thick mucus blockage in the trachea and air saculitis, leading to variable mortality. IBV often infects the reproductive system, damaging the oviduct, causing false pregnancy or "silent" layers, and significantly reducing egg production and hatchability (Rafique et al., 2024). Egg yolk peritonitis with secondary bacterial infections further impacts egg production. Control of IBV is crucial at the point of lay and immediately after hatching. Nephropathogenic IBV strains, including Holte, Gray, Australian T, and QX, cause significant kidney damage, particularly in young and meat-type chickens, leading to high mortality, weight loss, and growth retardation. In addition, co-infections with colibacillosis or other secondary infections exacerbate mortality rates (Khanh et al., 2018; Wang et al., 2024).

3. Current diagnostic approaches for IBV

Infectious bronchitis (IB) is classified as a multi-systemic infection in poultry, presenting clinical signs similar to other poultry diseases, making it challenging to differentiate between new IB variants due to their continuous evolution and emergence. Even modern techniques face difficulties in culturing IBV in embryonated eggs (Khan et al., 2023). Therefore, it is necessary to establish advanced diagnostic procedures that ensure accuracy using a series of laboratory techniques. Previously, the diagnosis of IBV mainly relied on clinical signs, virus isolation and antigen-antibody reactions. However, current molecular diagnostics offer faster and more accurate results, representing significant improvements (Machado et al., 2020). The use of several live attenuated vaccines in farms complicates the interpretation of diagnosis based on circulating field strains (Goraichuk et al., 2019). Additional factors-including the timing and pooling of samples, the stage of production and infection (whether active or post), and the use of appropriate diagnostic techniques-play a pivotal role in accurately assessing health and fertility in broiler breeders, as illustrated in Supplementary Table S1.

3.1. Gross and histopathology

Common post-mortem findings associated with IBV infection include the presence of an abnormal, underdeveloped blind sac protruding from the cloacal region. Certain strains, such as QX and D277, are known to cause cystic oviducts filled with watery substances, appearing as oval-shaped balloons containing approximately 0.5–1.0 L of fluid, or atrophic oviducts with large cystic dilations (Zhang et al., 2020). Birds infected with nephrogenic IBV strains often exhibit swollen or marbled kidneys with urate deposits in the tubules (Kuang et al., 2021). Additionally, deep pectoral myopathy, associated with renal lesions, is observed in broiler breeders infected by specific IBV variants. Chronic IBV infections may also result in ovarian lesions, including regressing follicles or false layers in laying hens, even though affected birds may appear normal and active.

Although histopathological examination is a rarely used approach for IBV diagnosis nowadays, it remains a valuable tool for research purposes, providing insights into the disease's pathogenesis. Histopathological examination of kidneys reveals congestion, hemorrhage, and multifocal necrosis. Additionally, there is lymphocyte and plasma cell infiltration in the urinary ducts, along with PAS (Periodic acid-Schiff)-positive granules and mitotic figures in tubular epithelial cells (da Silva et al., 2018). Tracheal histopathology varies with the severity of infection, commonly showing cilia loss, epithelial degeneration from columnar to desquamated cells, and glandular hypertrophy following IBV exposure (Bijanzad et al., 2013).

3.2. Viral isolation and identification

The most widely used method for isolating infectious bronchitis virus (IBV) involves cultivating the virus in embryonated chicken eggs, which provide a complex structure comprising the embryo and its associated membranes (chorioallantoic, amniotic, and yolk) (Guy, 2015; Hu, 2022; Banda and Yan, 2022). This technique relies on the replication of IBV in ciliated epithelial cells, achieving high viral titers within 1–2 days post-inoculation. Detection of IBV in inoculated eggs is typically confirmed using antigen tests or RT-PCR assays (Laconi et al., 2020; Guy, 2015).

While the allantoic route is commonly preferred due to the virus replicates extensively in the chorioallantoic membrane, resulting in

significant titers in the allantoic fluid, IBV can replicate effectively in embryonated chicken eggs regardless of the injection route (Banda and Yan, 2022; Sharma et al., 2020). To achieve a high virus titer, multiple passages in the allantoic fluid may be necessary, depending on the specific viral strain, which can prolong the diagnostic process. Inoculated eggs are typically opened after 5–7 days, and infected embryos are examined for characteristic signs such as curling and dwarfism, which are suggestive but not pathognomonic for IBV (Uddin et al., 2016; Villarreal, 2010; Bande et al., 2017).

Efforts have also been made to isolate IBV using various primary and secondary cell cultures, including Vero cells and chicken embryo kidney fibroblasts. However, a major limitation of cell culture techniques for IBV isolation is that not all IBV strains readily adapt to cell culture (Laconi et al., 2020; Guy, 2015). Even for strains that can adapt to cell culture, such as M41, Iowa 97, and NZ, initial isolation in embryonated chicken eggs is often necessary to facilitate virus propagation.

Although virus isolation provides valuable insights, it is a labour-intensive process with several limitations. As noted by Bhuiyan et al. (2024) and Callison et al. (2006), isolation often requires at least three passages in embryonated egg, with a turnaround time ranging from several days to weeks, limits its use for urgent diagnostics. Moreover, while virus isolation can detect viable virus across all serotypes, strains with low viability or low titres may be missed, resulting in variable sensitivity of subsequent PCR testing depending on the sample quality (Gallardo, 2021).

3.3. Serological study

Enzyme-linked immunosorbent assay (ELISA) is a suitable technique for monitoring the immune status of birds, assessing vaccine efficiency, evaluating viral challenges, and determining the longevity of viral infections (Legnardi et al., 2020; Bhuiyan et al., 2024). Several commercial ELISA kits are available for detecting IBV-specific antibodies. In unvaccinated flocks, the detection of positive IBV titers in serology can confirm a field challenge. However, in IBV vaccinated flocks, detecting a field challenge is more challenging, as vaccinated birds exhibit a certain level of antibody titers (Van Leerdam, 2011; Bhuiyan et al., 2021a,b). To optimize the utility of ELISA test, it is important to establish farm-specific baselines that consider factors such as vaccination programs, the geographical distribution of IBV strains, outbreak histories, the specific vaccines used, and the types of birds being inspected. These factors, combined with periodic flock serological reporting, can help establish a serological history to decide whether serological results are normal or abnormal.

Antigen rapid tests (ART), also known as lateral flow tests, offer a faster alternative for IBV detection. These tests detect specific proteins (antigens) associated with IBV and utilize respiratory tract swabs from chickens (Liu et al., 2019). ARTs provide quick results within 15–30 min and are suitable for use in diverse settings without requiring specialized equipment. However, their sensitivity and specificity can vary, potentially leading to false-negative or false-positive results, which may limit their reliability.

The virus neutralization (VN) test remains one of the most precise methods for identifying and distinguishing between IBV serotypes. The principle of viral neutralization assays is to evaluate the number of virus-neutralizing antibodies in serum samples against a known infectious virus or to use reference antisera or monoclonal antibodies to identify an unknown virus. In the VN test, diluted IBV is mixed with antiserum and subsequently inoculated into embryonated chicken eggs. Following incubation, the samples are injected into the eggs, and pathogenic changes in the embryos are observed after seven days to determine the VN titer. Due to the rapid mutation rates of IBV, identifying prevalent serotypes in specific regions is essential, making the VN test an indispensable tool for guiding vaccine selection. Proper vaccine selection is crucial, as different IBV antigenic types do not provide cross-protection.

One of the limitations of serologic cross-reactivity is that it is not a reliable indicator of IBV, as reported by Gelb et al. (2005). Wang et al., 2024 noted that ELISA could lead to false-positive results or complicate the differentiation between vaccine-induced and natural infections. Even, both ELISA and hemagglutination inhibition tests often lack the specificity required to differentiate closely related IBV serotypes. Jiang et al. (2020) pointed out that shared antigenic epitopes among serotypes lead to cross-reactivity, reducing the precision of serological detection.

3.4. Molecular diagnosis

Current molecular biology techniques, such as RT-PCR, nested and semi-nested RT-PCR, and real-time RT-PCR, are also employed for the rapid and sensitive detection of IBV in clinical samples. The qRT–PCR method amplifies viral RNA either directly through one-step RT-PCR or following cDNA synthesis in a two-step RT-PCR process. In RT-PCR, amplified PCR products can be further analyzed to identify genetic types and strains of IBV through gene sequencing and phylogenetic analysis of targeted DNA fragments (Saito et al., 2022; Assanov et al., 2023). This approach enables the detection of all IBV serotypes by targeting conserved regions, such as the S and N genes, as well as untranslated regions (UTR), using specific primers (Bande et al., 2017; Okino et al., 2018). Ameen et al. (2022) reported using a multiplex real-time RT-PCR assay to differentiate between classical and variant strains of avian IBV. Using qRT-PCR, IBV can be distinguished between mass-type and non-mass-type to obtain the comprehensive data about the specific types of IBV present. Moreover, further analysis is performed using techniques like RFLP (Restriction Fragment Length Polymorphism) that involves cutting the viral DNA into fragments with specific enzymes (Sruthy et al., 2022), sequencing (Sruthi and Prakash, 2019), and microsphere-based assays (Roh et al., 2013), allowing for detailed characterization and comparison of different viral IBV strain. Genotype-specific RT-PCR assays are used for the rapid molecular typing of vaccine strains and field variants, focusing on the S1 protein's cleavage site identified by host cell serine protease. (Leow et al., 2018). Gene sequencing and phylogenetic analysis of amino acid sequences are critical for understanding the conserved and non-conserved domains of IBV, aiding in epidemiological investigations (Spielman et al., 2019; Parvin et al., 2021). Moreover, PCR is widely recognized for its high sensitivity and specificity in detecting known genotypes of IBV (Mo et al., 2020) (summarized in Supplementary Table S2). However, the effectiveness of this method can be compromised if the designed primers lack conserved regions specific to certain genotypes. This limitation may hinder the ability to differentiate between serotypes, thereby reducing PCR's accuracy in epidemiological investigations (Valastro et al., 2016). Furthermore, the emergence of novel IBV genotypes poses a significant challenge to traditional techniques like PCR, which rely on prior genetic knowledge of the virus. Emerging strains with significant genetic divergence from known genotypes often evade detection, complicating diagnostic efforts and undermining the utility of PCR in rapidly evolving epidemiological scenarios (Zhao et al., 2021). Similarly, virus isolation methods are subject to limitations due to the potential for cross-reactivity with other avian respiratory viruses. This issue is particularly pronounced in environments with mixed infections, where cross-reactivity reduces the specificity of the diagnostic approach (de Wit et al., 2011).

3.5. Other less commonly used diagnostic approaches

The ciliostasis test is a widely used method to evaluate the pathogenicity of IBV and the effectiveness of specific vaccination programs. It measures the ciliary activity of the tracheal mucosa as an indicator of the level of protection against pathogen entry. In this test, the level of protection is determined by estimating the percentage of the tracheal epithelial surface exhibiting ciliary movement following challenge or infection. This percentage is usually categorized into classes of 25% or 50%, corresponding to a score of 0–4 or 0–1, respectively, for each

tracheal segment. Various studies have recommended that the protection scoring method (ciliostasis) and sampling should involve at least 10 tracheal rings from each of 10 chickens per group, utilizing a 5 class (0–4) scoring system (Shao et al., 2020; Khataby et al., 2016). A chicken is considered protected if more than 50% of the epithelium of the TOCs exhibits ciliary movement after the challenge (Hennion, 2015).

In situ hybridization (ISH), while less commonly used due to advancements in modern technologies, remains a valuable method for the specific identification of IBV genomic RNA. ISH employs recombinant DNA probes to localize specific nucleic acids within histological sections for IBV detection (Veselinyová et al., 2021). Universal DIG-labeled probes can detect all IBV serotypes by constructing complementary mRNA sequences of the membrane gene. ISH with RNA-labeled riboprobes demonstrates higher sensitive than DNA probes but require stringent hybridization conditions (Young et al., 2020).

Electron microscopy primarily provides detailed morphological information about coronaviruses. Positive cultures confirm the image-based structure of coronaviruses, which appear pleomorphic with spike projections when subjected to negative staining with phosphotungstic acid (Prasad et al., 2020). Various studies have reported cytopathological changes and tracheal ciliostasis in tracheal cultures as early as three days PI (Seifi1 et al., 2015; Khataby et al., 2016).

Different RNA molecules have been studied by evaluating their "fingerprints" through specific RNase digestion and oligonucleotide determination (Borgelt and Wu, 2023). RNase T1 fingerprinting is a computational assay that compares large RNA molecules by resolving oligonucleotides generated through ribonuclease (RNase) digestion using two-dimensional gel electrophoresis. This method allows for the comparison of RNA fingerprints with those of known field strains.

The REFLP method is used for IBV genotyping and comparison with different known IBV strains. This assay facilitates rapid identification of new variants or virus typing following RT-PCR amplification (Lin and Chen, 2017). RFLP patterns are normally compared to the patterns of several known IBV serotypes based on their unique banding patterns observed in gel electrophoresis during RE digestion (Montassier, 2010). However, there are limitations in field studies using RFLP-genotyping for new isolates, as it may not always provide accurate information about the true antigenic nature of these IBV isolates.

4. Current challenges in IBV detection

The detection of avian coronaviruses presents several challenges, including.

a. **Diverse serotypes and genotypes**: Avian coronaviruses, such as IBV, exhibit a high degree of genetic variability, leading to the emergence of numerous serotypes and genotypes (Marandino et al., 2023). This diversity complicates diagnostic efforts, as different strains may exhibit varying levels of virulence and tissue tropism.
b. **Mutations, recombination's and deletions**: The high mutation rate of avian coronaviruses, coupled with recombination events and genomic deletions, contributes to the rapid evolution and emergence of new strains (Xing et al., 2022). This genetic plasticity poses challenges in designing diagnostic assays capable of accurately detecting all viral variants.
c. **Tissue tropism and multi-systemic infection**: Avian coronaviruses can infect multiple organ systems, including the respiratory, reproductive, and renal systems (Quinteros et al., 2022). This broad tissue tropism necessitates diagnostic tests capable of detecting viral RNA or antigens in a wide range of sample types, such as mucus, faces, or tissue (Najafi Fard et al., 2021).
d. **Limited sensitivity and specificity of conventional techniques**: Traditional diagnostic techniques, like PCR, virus isolation, and serological assays, have limitations in terms of sensitivity, specificity, and turnaround time (Dronina et al., 2021). These techniques may fail to detect low levels of viral RNA or antigens, leading to false-negative results or misdiagnosis.
e. **Inability to differentiate immune status from active infection**: Serological assays, while useful for assessing immune status and predicting future disease outbreaks, cannot differentiate between active infection and past exposure to the virus (de Wit et al., 1997; Fox et al., 2022). This limitation hinders the accurate diagnosis of ongoing outbreaks and the implementation of appropriate control measures.
f. **Need for rapid and precise diagnosis**: In commercial poultry farming, timely and accurate diagnosis of avian coronavirus infections is crucial for implementing effective control measures and minimizing economic losses (Liebhart et al., 2023). Delays in diagnosis can result in the rapid spread of the virus within flocks and across regions.

5. Prospect of biosensor-based detection of IBV

Biosensors are analytical devices that integrate a biological sensing element with a transducer to produce a measurable signal proportional to the concentration of the target analyte (Naresh and Lee, 2021). The application of biosensors in the detection of IBV offers rapid, sensitive, and specific detection of the virus. IBV detection biosensors can target different molecules associated with IBV, such as viral proteins or viral nucleic acids (RNA), which is crucial for early diagnosis and surveillance of IBV infections (Yang et al., 2020). These devices, based on electrochemical, optical, piezoelectric, and magnetic sensors, can accurately target viral proteins or RNA, enabling early diagnosis. The primary challenges include achieving high sensitivity and specificity, ensuring compatibility with poultry production sample types, and the potential for point-of-care applications. Integrating these biosensors with Internet of Things (IoT) platforms and data analytics tools can enable real-time monitoring of IBV prevalence and transmission dynamics, enhancing overall disease management and control strategies.

Various transduction methods, including electrochemical, optical, or mass-based techniques, can be employed to detect this signal. Signal amplification methods may be utilized to enhance sensitivity, especially when dealing with low concentrations of the target virus. The collected data is then analyzed to determine the presence and concentration of the virus in the sample. A biosensor's schematic diagram shows a transducer, a signal processing unit, and a bioreceptor for the detection of the relevant analyte in spatial contact as shown in Fig. 1.

Developing a DNA biosensor for virus detection necessitates a thorough understanding of the specific virus's genetic material, along with considerations for the stability and reproducibility of the biosensor. Ongoing research in the field may also introduce new technologies and improvements to existing biosensor designs. Different types of rapid diagnostic biosensor assays have been studied for detecting avian influenza (AI) (bird flu), such as glycan-conjugated nanoparticle-based sensors (Büyüksünetçi and Anık, 2023; Zheng et al., 2017), fluorescence resonance energy transfer (FRET) and impedimetric biosensors (Hushegyi et al., 2015). Moreover, different types of field effect transistor (FET) based biosensor, silver nanoparticle fluorescence-based sensors, immunogold biosensors, and glycan-coated gold nanoparticles are used to distinguish between 14 serotypes of AI (Zheng et al., 2017; Hideshima et al., 2013).

Virus-like particles (VLPs) have been extensively researched for transporting various compounds such as medicines, peptides/proteins, RNA/DNA, antibodies, and vaccines. Novel modern technologies have been developed for detecting poultry disease using DNA biosensors that convert biological signals or bio-receptors to detectable electrical signals (Du and Zhou, 2018; Adam et al., 2023; Huang et al., 2022). Nowadays, these approaches can be applied to various types of poultry diseases due to their higher sensitivity and specific detection of virus serotypes, which are comparable in equal sensitivity to RT-PCR results (). VLPs also serve as antigen nanocarriers and adjuvants to immune cells, aiming to

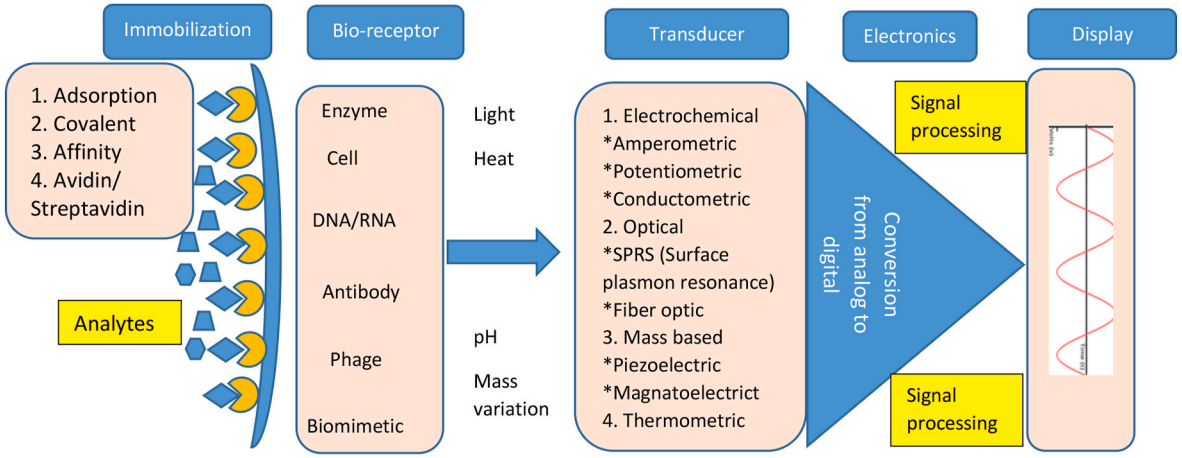

Fig. 1. A biosensor's schematic diagram shows a transducer, a signal processing unit, and a bioreceptor for the detection of the relevant analyte in spatial contact. These elements interact with analytes, convert the interaction into quantifiable signals, and display the output as digital data.

elicit a protective humoral immune response (Abusalah et al., 2023). The cell surface protein S, binding to the receptor, can trigger an immune response (Bellavite et al., 2023). Chen et al. (2016) described a conventional approach utilizing avian coronavirus (CoVs) VLPs based on the S protein, employing 100-nm gold nanoparticles with an optimized concentration of viral proteins. This study led to the spontaneous formation of proteins inducing the assembly of virus-like nanostructures with viral antigens coating the fundamental particulate. The results from this study validate the successful preparation of synthetic VLPs (sVLPs) through nanoparticles, demonstrating an inherent tendency for protein coating (Wang et al., 2024).

5.1. DNA biosensor

A DNA biosensor designed for virus detection is a diagnostic tool that employs DNA-based recognition components to selectively identify the presence of a specific virus. The core principle of DNA biosensors lies in their capacity to detect the hybridization of complementary DNA strands (Choi and Yoon, 2023; Ribeiro et al., 2020). This is achieved by immobilizing a specific DNA strand on the biosensor's surface, which

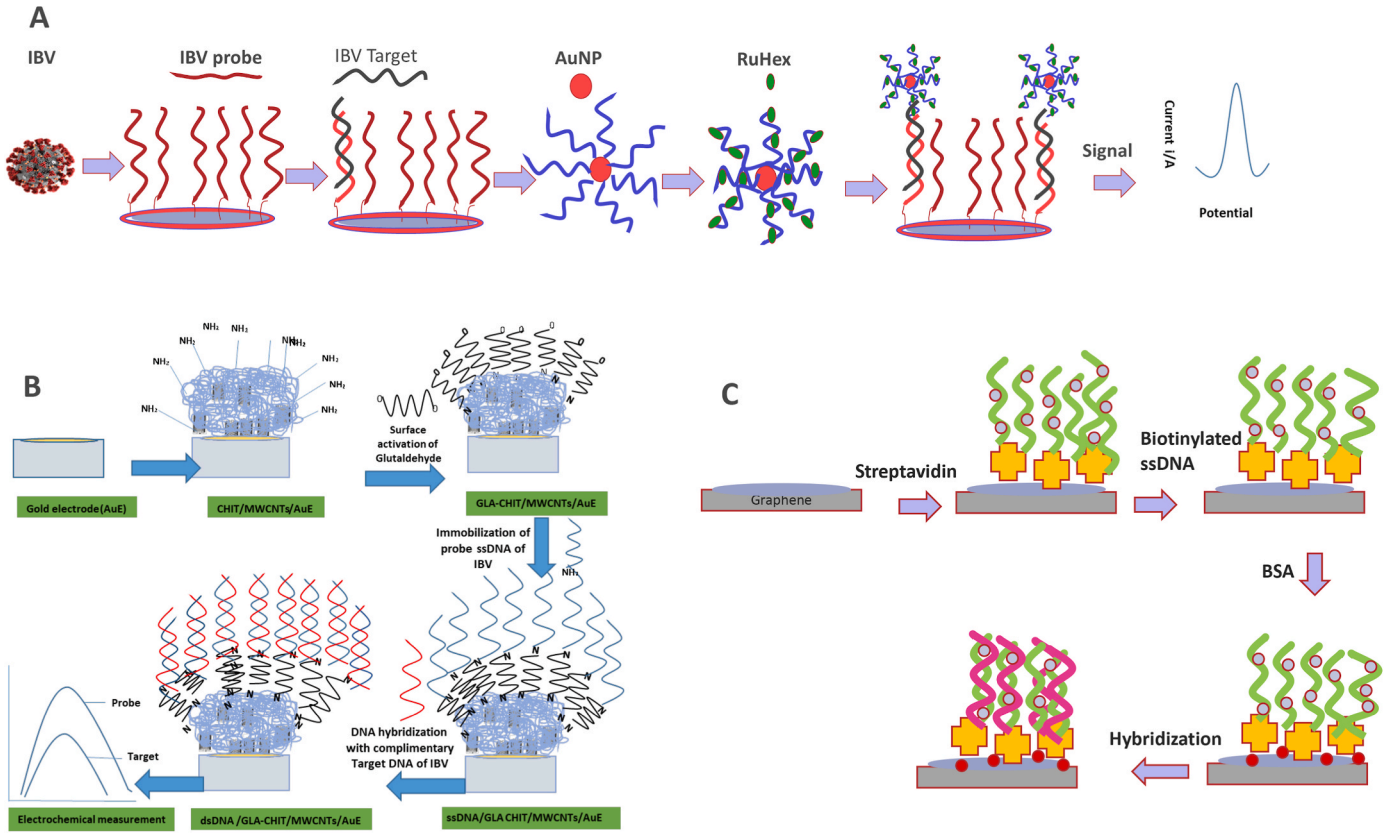

Fig. 2. A) Schematic representation of the fabrication principles of immobilization and hybridization with activated ssDNA probe and dsDNA for IBV detection, B) Diagram of the label-free electrochemical test for measuring the coronavirus IBV H120 strain using the comparable substitution effect and signal amplification enhanced by AuNPs, C) Detailed explanation of the experimental setup and assay design for identifying a synthetic SARS-CoV-2 DNA sequence with Streptavidin-modified FPCB-implemented graphene electrode and biotinylated ssDNA capture sequence functionalization with BSA blocking and target DNA capture.

then interacts with its complementary strand in the sample (Mukherjee et al., 2022). The mechanism of DNA biosensors is based on biological interactions between the target molecule and the bioreceptor, which either generates or consumes ions or electrons, leading to alterations. The changes resulting from the reaction can be transformed into electrical signals, which are then amplified and measured by the transducer. The overall process of a DNA biosensor for virus detection begins with the selection of a DNA probe, which involves designing a brief DNA sequence that complements a specific region of the virus's genetic material. This DNA probe is then fixed onto the surface of a sensor for immobilized onto a transducer surface, typically composed of materials like gold, silicon, or glass. When a virus-containing sample is introduced to the biosensor, the target genetic material (RNA or DNA) binds to the complementary DNA probe, forming a stable duplex. This interaction generates a measurable signal that reflects the presence of the virus (Babaei et al., 2022). A schematic representation of the fabrication principles of immobilization and hybridization using an activated ssDNA probe and dsDNA for different DNA biosensors techniques in IBV detection is illustrated in Fig. 2.

IBV, which exhibits a considerable number of serotypes or genotypes, can be effectively detected using DNA biosensors designed to detect different variations in serotypes. The design principles for biosensors targeting different viral strains, such as IBV and AI, often share common elements. Both benefit from the integration of biological recognition elements, advanced sensor technologies, and artificial intelligence for data analysis, adaptability, and improved detection accuracy. The key is to employ the biosensor to the specific characteristics of each virus while leveraging common strategies for effective detection.

In the current context of increasing poultry production and future growth prospects, it is crucial to control and reduce the time between early infection and diagnosis. Therefore, biosensors and surveillance technologies should be applied for the rapid, on-site diagnosis of chicken diseases at the farm level (Abdel-Haleem et al., 2021; Ang et al., 2023). In the realm of electrochemical biosensors, nanomaterials are currently of significant interest for detecting IBV with others coronavirus and detection sensitivities (Supplementary Table S3).

A label-free electrochemical approach was developed, integrating the equivalent substitution effect with AuNPs-assisted signal amplification. By targeting distinctions in the S1 protein among various strains of the IBV, a specific DNA sequence was designed to selectively recognize H120 RNA, forming a double-stranded structure through DNA-RNA hybridization. This approach achieved an effective detection range spanning from $1.56e^{-9}$ to $1.56e^{-6}$ μM, with a detection limit of $2.96e^{-10}$ μM, enabling selective quantification of the IBV H120 strain, demonstrating significant potential for applications in IBV detection within the realms of vaccine research and avian infectious bronchitis diagnosis (Yang et al., 2020).

A recent study developed an electrochemical DNA biosensor using a gold electrode coated with a nanocomposite of chitosan (CS), multi-walled carbon nanotubes (MWNTs), and highly reactive glutaraldehyde (GLU). The biosensor targets the Orf gene of IBV, enabling rapid and accurate detection of the viral disease in poultry. Optimal conditions were determined through cyclic voltammetry (CV) and differential pulse voltammetry (DPV) with methylene blue as the redox indicator. The biosensor successfully detected target DNA in the concentration range of 2.0 x 10^{-12} to 2.0 x 10^{-5} molL-1, with a limit of detection (LOD) and limit of quantitation (LOQ) of 2.6 nM and 0.79 nM, respectively (Bhuiyan et al., 2021).

Limited research has been conducted on biosensors for the diagnosis of IBV, in contrast to the substantial body of work dedicated to SARS-CoV-2. Despite the divergent characteristics of these viruses, the genetic similarities among various coronaviruses suggest that methodologies developed for SARS-CoV-2 could be adapted for novel DNA biosensor approaches for IBV. One study reported the development of an electrochemical biosensor chip using G, capable of selectively recognizing SARS-CoV-2 RNA (N gene) (Alafeef et al., 2023). This chip immobilizes highly specific ssDNA-capped gold nanoparticles (AuNPs) probes for the viral N gene onto G-coated filter paper. The inclusion of AuNPs enhances the electrochemical response by facilitating electron transfer and providing a large surface area for the ssDNA probe. The G-ssDNA-AuNPs platform can deliver results in just 5 min with a handheld reader, offering POC testing capabilities that are particularly valuable in resource-limited settings. Similarly, Zhao et al. (2021) developed an ultra-sensitive sandwich electrochemical sensor using calixarene-functionalized graphene and SARS-CoV-2-targeted RNA, achieving a LOD as low as 200 copies/mL for clinical samples. This sensor, when paired with a smartphone, can effectively detect SARS-CoV-2 without the need for RNA amplification. The detection process is sensitive, accurate, and rapid, providing a cost-effective and straightforward approach to point-of-care diagnosis. Additionally, the combination of Rolling Circle Amplification (RCA) and Catalytic Hairpin Assembly (CHA) in nucleic acid amplification strategies offers an effective means to enhance electrochemical signals with precision.

An electrochemical biosensor for SARS-CoV-2 RNA targeting the S or N gene was developed utilizing rolling circle amplification (RCA) (Chaibun et al., 2021). This technique enables virus detection at an exceptionally low level of one copy/μl within a 2-h timeframe, using differential pulse voltammetry (DPV). The integrated design harnesses the synergistic strengths of RCA and catalytic hairpin assembly (CHA), resulting in highly sensitive and accurate amplification of electrochemical signals, thereby enhancing the efficiency of SARS-CoV-2 RNA detection. Similarly, an electrochemical sensor utilizing CHA and terminal deoxynucleotidyl transferase (TdT)-induced polymerization was made available for the detection of ORF1ab gene of SARS-CoV-2 (Peng et al., 2021). This approach involves the formation of a Y-type DNA structure through the interaction of the target gene with hairpins HP1 and HP2, which subsequently activates TdT-induced polymerization. This process generates a substantial amount of long single-stranded DNA products in the deoxyribonucleotide triphosphate (dNTP) pool. During this step, many Ru $(NH3)_63+$ molecules adsorb onto the DNA phosphoric acid backbone through strong electrostatic interactions, resulting in significantly enhanced electrochemical signals, enabling sensitive monitoring of SARS-CoV-2. In another advancement, Kashefi-kheyrabadi et al. (2022) developed an electrochemical sensor that does not involve nucleic acid amplification. In their detection process, both the S and ORF1ab genes of SARS-CoV-2 can be simultaneously detected within 1 h, with LOD as low as 5.0 and 6.8 ag/μl, respectively. This method offers a rapid and sensitive means for the detection of SARS-CoV-2 genetic material without the need for amplification steps. Moreover, an innovative technique combined CRISPR/Cas13a technology with an electrochemical biosensor was implemented for the detection of SARS-CoV-2 RNA (Heo et al., 2022). In this approach, the single-stranded RNA (ssRNA) probe is recognized by the SARS-CoV-2 RNA phase, leading to the formation of the Cas13a-crRNA complex. This complex is then introduced into the reporter RNA (reRNA)-coupled electrochemical sensor, activating RNase, which subsequently cleaves reRNA. During this process, the redox molecules released by reRNA induce changes in the current, enabling sensitive detection of SARS-CoV-2 RNA. Importantly, the amplification-free sensors developed by the team allow for ultra-low concentration testing of SARS-CoV-2 RNA, holding promise for on-site and rapid diagnostic testing of COVID-19.

5.2. Immunosensors

The principle of immunosensors involves their function as biosensing devices that employ affinity ligands to detect specific targets by linking immunochemical reactions to appropriate transducers (Siew et al., 2021; Büyüksünetçi and Anık, 2023). The immunosensor, a specialized type of biosensor, utilizes solid-state affinity mechanisms to detect a targeted analyte, specifically an antigen (Ag), by orchestrating the creation of a stable immunocomplex through the interaction between the

antigen and an antibody serving as a capture agent (Ab) (Bao et al., 2023). This interaction ultimately generates a measurable signal with the assistance of a transducer. At the core of every immunosensor lies the crucial principle of molecular recognition, where antibodies exhibit specificity in forming stable complexes with antigens. This principle closely mirrors the methodology employed in immunoassays. The categorization of immunosensors is contingent upon the specific detection principles applied in their design. A schematic representation of the fabrication principles of an immunobiosensor, employing antigen-antibody interactions for various techniques of IBV detection, is shown in Fig. 3. Research on immunosensors for detecting IBV is currently limited, with a significant amount of research concentrated on the SARS, which is closely related to IBV. However, there is a growing interest among researchers to develop and explore the intricacies of immunosensor applications in the context of IBV and related viruses. Summary of target viruses and detection limits for immunobiosensors based on various nanomaterials as shown in Supplementary Table S4. Recently, scientists have devised a rapid, cost-effective, and highly sensitive biosensor for the detection of IBV using molybdenum disulfide (MoS2). This two-dimensional nanosheet, combined with a dye-labeled antibody (Ab), demonstrates robust fluorescence-quenching capabilities (Weng and Neethirajan, 2018; Janik-Karpinska et al., 2022). After optimizing the assay conditions, the immunosensor exhibited outstanding sensitivity, accurately measuring M1 EID50 per mL. Additionally, it displayed specificity within a dynamic linear response range of 10^2–10^6 EID^{50} per mL for IBV standard solutions. Notably, the optimized immunoassay successfully identified IBV in spiked chicken serum, yielding reliable results and showcasing its potential for on-farm detection.

A rapid immunochromatographic strip (ICS) was developed for identifying IBV infection using monoclonal antibodies targeting S and N proteins (Liu et al., 2019). The ICS showed significant specificity for IBV antigens, effectively distinguishing between various genotypes and emerging variants, and other avian respiratory viruses. With a detection limit of $10^{4.4}$ 50% embryo-infective dose, this ICS represents a novel approach for the swift identification of IBV infection, completing the test within 10 min. Notably, it is the first instance of IBV antigen detection using an ICS. Comparative analysis with the reference RT-PCR test revealed that the ICS could detect antigens as early as 1-day post-infection (dpi) from throat swab samples and 5 dpi from cloacal swab samples, demonstrating a detection limit ranging from $10^{4.5}$ to $10^{4.8}$ 50% embryo-infective dose (EID^{50}). The ICS showed superior performance compared to liquid-phase blocking ELISA, with broad-reactive mAbs exhibiting cross-reactivity against various IBV types. Further investigations are needed to validate the antigen–antibody reaction against other globally common strains.

Moreover, a chiral zirconium quantum dots (Zr QDs) was introduced as a novel class of nanocrystals for the detection of coronavirus (Ahmed et al., 2018). Using L (+)-ascorbic acid as a ligand, they characterized the QDs, revealing fluorescence and circular dichroism properties. The synthesized QDs displayed fluorescence and circular dichroism properties, with peak wavelengths observed at 412 nm and 352 nm, respectively. Conjugation of the QDs with anti-IBV antibodies enabled biosensing with a detection limit of 79.15 EID_{50}/50 μL, demonstrating the potential of nanocrystals in virological diagnostics.

Introduced silicon photonic probes based on the broad-band Mach-Zehnder interferometry for directly immersible immunosensors was used, eliminating the need for microfluidics and pumps (Angelopoulou et al., 2022). The analytical potential of these photonic probes was demonstrated by detecting antibodies against SARS-CoV-2 in human serum samples. One MZI was functionalized with the Receptor Binding Domain (RBD) of the SARS-CoV-2 Spike 1 protein, while the other was

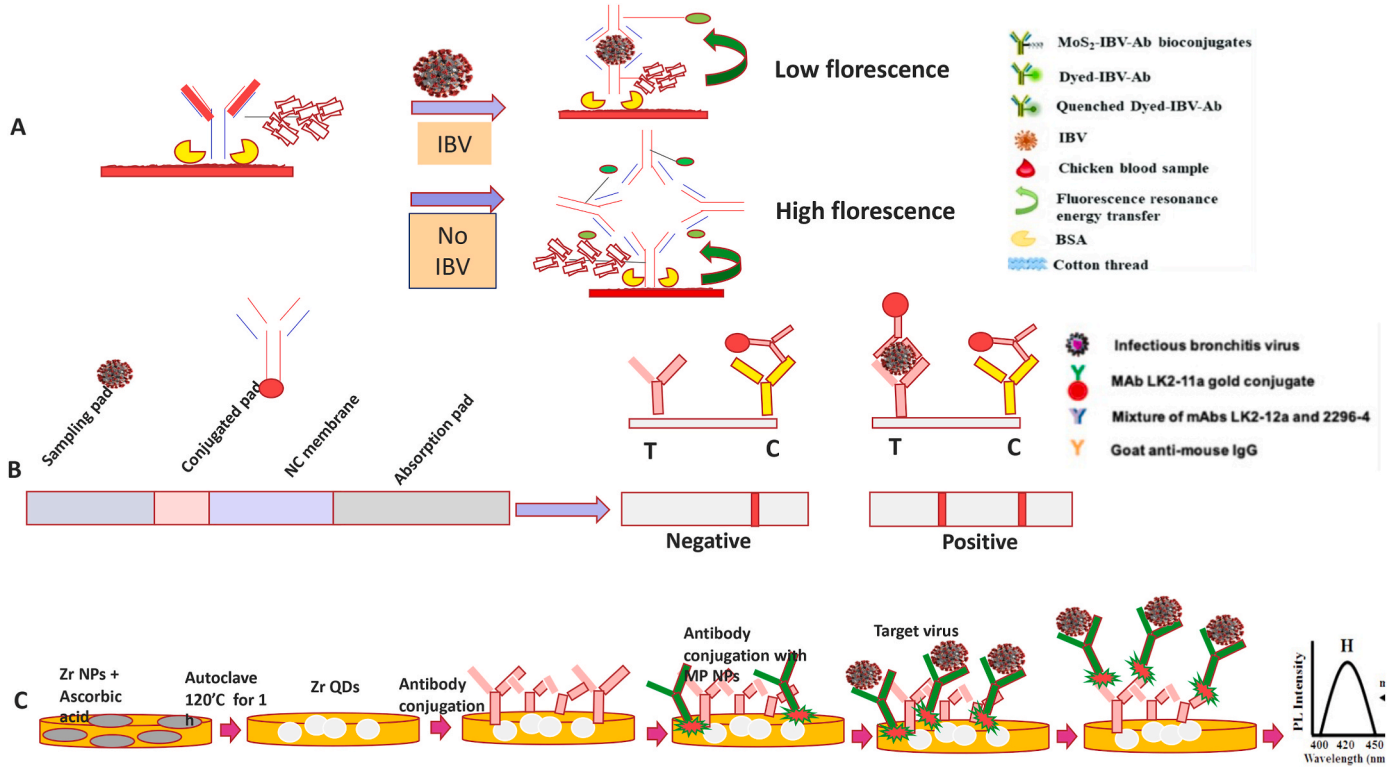

Fig. 3. A) Illustration of a single-step homogeneous immunoassay conducted on a cotton thread, utilizing MoS2-based Förster resonance energy transfer (FRET) to identify the presence of Infectious Bronchitis Virus (IBV). B) Diagram illustrating the fabrication process, depicting the principles of immobilization and hybridization involving an activated single-stranded DNA (ssDNA) probe and double-stranded DNA (dsDNA). C) A sensor design scheme for synthetic chiral zirconium quantum dots (Zr QDs), utilizing L (+)-ascorbic acid as both a surface and chiral ligand for the QDs. The synthesized QDs then intricately combined with antibodies specific to IBV, resulting in the formation of an immunolink upon the presence of the target analyte. Finally, the scheme incorporated magneto-plasmonic nanoparticles (MPNPs) conjugated with antibodies targeting IBV, adding another layer of sophistication to the sensor design.

functionalized with bovine serum albumin as a reference. Immersing the biofunctionalized probes in human serum samples and then in goat anti-human IgG Fc specific antibody solution revealed a detection limit of 20 ng/mL using a humanized rat antibody against SARS-CoV-2 RBD. The system effectively differentiated between non-infected/non-vaccinated and vaccinated individuals, with antibody levels correlating well with ELISA results, showcasing its utility in rapid point-of-care testing. In response to concerns about false negatives in molecular detection via oral swabs during the SARS-CoV-2 epidemic, Wen et al. (2020) developed a lateral flow immunoassay (LFIA) strip for serological testing. The LFIA is user-friendly, completes within 15–20 min, and offers stability and reproducibility, making it a valuable tool for low-resource settings and seroprevalence studies.

Moreover, a sensitive LFIA using lanthanide-doped polystyrene nanoparticles (LNPs) was developed to detect anti-SARS-CoV-2 IgG in human serum (Chen et al. (2020). The assay captures specific IgG using a recombinant nucleocapsid phosphoprotein of SARS-CoV-2 and uses mouse anti-human IgG antibodies labeled with self-assembled LNPs. The detection process is completed within 10 min, meeting clinical diagnostic reagent requirements. This assay demonstrates rapid and sensitive detection, facilitating positive identification in suspicious cases. Similarly, an impedimetric immunosensor was employed for the rapid detection and monitoring of SARS-CoV-2 antibodies in human serum, prompted by the COVID-19 pandemic (Shoute et al., 2023). This device uses an interdigitated microelectrode array (IMA) and conjugation chemistry to immobilize the spike protein, facilitating the binding of anti-spike antibodies. The sensor has a LOD of 0.4 BAU/ml, comparable to commercial assays. Las but not least, an immunosensor was used for the rapid detection of the spike protein from SARS-CoV-2 (Vásquez et al., 2022). The device utilized magnetic beads conjugated with anti-spike and anti-ACE2 antibodies, achieving a sensitivity of 0.83 μA (mL/μg) and a detection limit of 22.5 ng/mL. It successfully identified spike protein in commercial buffers, pseudovirions, isolated viral particles, and nasopharyngeal swabs from infected patients.

5.3. Optic biosensors

The principle of an optical biosensor is to measure the concentration of a substance by monitoring changes in the characteristics of light, such as intensity, wavelength, or polarization, as it interacts with the analyte (John et al., 2023; Courtneyet al., 2021; Sharma et al., 2021). These changes arise from interactions between the analyte and the receptor, which modify specific light properties such as fluorescence, absorption, or refractive index (Lin et al., 2023; Ambartsumyan et al., 2020). The intensity of the signal generated corresponds to the analyte concentration, enabling precise quantification.

Optical methods are extensively used for virus detection, leveraging techniques such as colorimetry, fluorescence, Raman scattering, chemiluminescence, plasmon resonance, dynamic light scattering, and plasmonic approaches, as illustrated in Fig. 4. While some of these methods involve complex fiber-optic structures, the demand for accessible and efficient fiber-optic sensors, particularly those utilizing standard optic materials and devices, is growing. These biosensors are highly regarded for their exceptional sensitivity and precision in analyte detection. Among these, Surface-Enhanced Raman Scattering (SERS)-encoded nanoparticles, commonly known as SERS tags, have emerged as a superior alternative to traditional colloidal gold. SERS tags comprise key components such as Raman reporter dyes adsorbed onto gold or silver nanoparticle substrates and specific antibodies that bind to their respective targets. These advancements highlight the critical role of optical biosensors in enhancing virus detection and advancing diagnostic technologies.

Recent advancements in optical biosensors for detecting viral infectious diseases in poultry and humans, including IBV, SARS, and SARS-

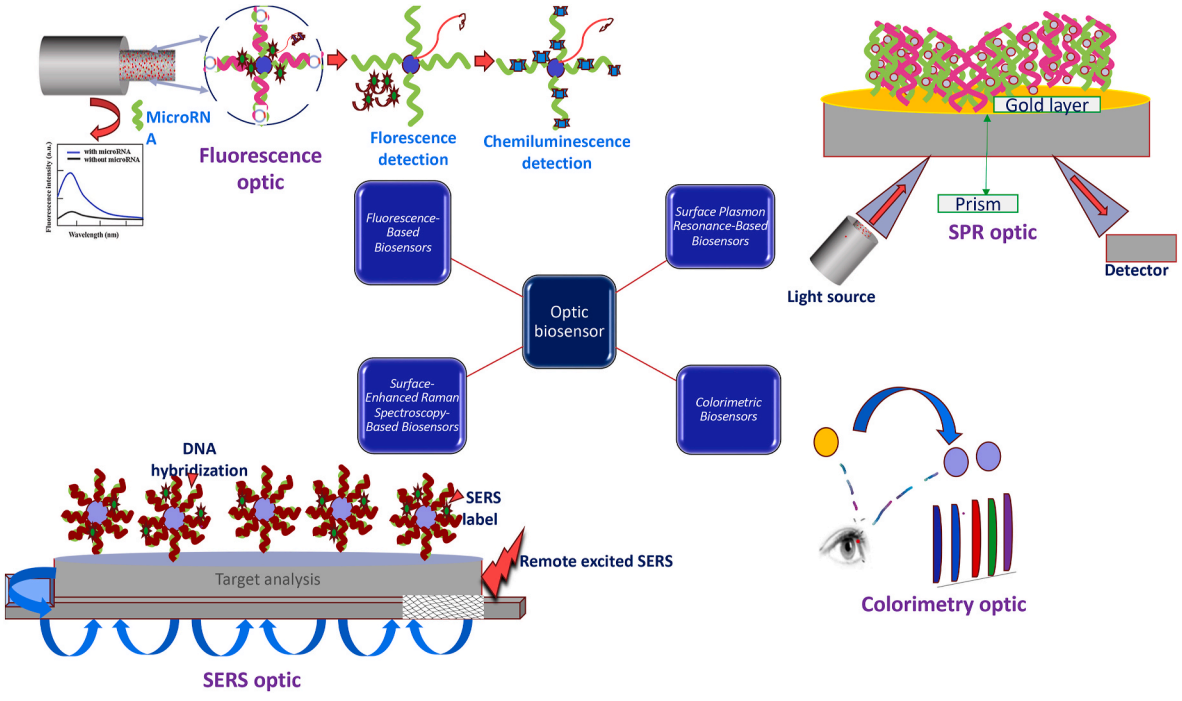

Fig. 4. Illustration of an advanced optical biosensor: fluorescence, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS) and colorimetric detection for microRNA and DNA hybridization. Key components of the diagram include fluorescence optics, which used fluorescence-based biosensors for the detection of microRNA via fluorescence emission. The inset diagram shows fluorescence spectra for the detection of microRNA with and without targets. Surface plasmon resonance (SPR) optics, which includes a prism and detector setup to measure refractive index changes caused by target binding at the sensor surface. Surface-enhanced Raman spectroscopy (SERS) uses labeled SERS probes for enhanced signal detection via Raman scattering, including a remote excitation setup. The final part of colorimetric detection is using visual cues or spectral changes for target identification, supported by colorimetric biosensors. This integrated design demonstrates the constructive interaction of fluorescence and SERS.

CoV-2, are continuously expanding through ongoing research (John et al., 2023; Xu et al., 2022). These advancements cover various sensor systems, including calorimetric, fluorescence, chemiluminescence, surface plasmon resonance, and photonic transduction methods. The paper discusses the prospects and commercialization of optical sensors for viral detection, providing a background for researchers to develop innovative approaches for point-of-care optical diagnostic sensing systems for various pathogens, including contagious viruses.

A novel refractive index-based optically transparent biosensor device has been developed for the rapid detection of COVID-19. This device uses a D-shaped gold-coated surface plasmon resonance configuration and is specifically designed for detecting IBV with refractive indices of −0.96, −0.97, −0.98, −0.99, and −1 (John et al., 2023; Balamurugan et al., 2023). The biosensor has a maximum wavelength sensitivity of 40, 141.76 nm/RIU and a minimum insertion loss of 2.9 dB. It's simple design, high sensitivity, and low losses make it effective in detecting COVID-19. Another study represented a Q-Factor, ultrasensitive THz refractive-index-based metamaterial biosensor for coronavirus detection at Electronic Infusion Device concentrations, featuring polarization insensitivity, angular stability, and near-perfect absorption (Das et al., 2020).

Optical methods are widely used for virus detection, employing techniques such as colorimetry, fluorescence, Raman scattering, chemiluminescence, plasmon resonance, dynamic light scattering, and plasmonic approaches. Fiber optic biosensors, constructed from glass or polymer fibers, utilize various measurement mechanisms to modulate the intensity, phase, or spectra of the optical signal. A biophotonic sensor designed for the specific detection of SARS-CoV-2 immunoglobulin G (IgG) antibodies relies on a single-mode telecommunication fiber, demonstrating the capability to selectively detect IgG antibodies in less than 1 min using a sample volume as small as 5 μl (Szczerska et al., 2023).

Spectroscopic techniques have also been explored for virus detection, including nuclear magnetic resonance spectroscopy, near-infrared (NIR) spectroscopy, Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), and molecular fluorescence spectroscopy (Santos et al., 2017). Non-optical spectroscopic approaches, such as nuclear magnetic resonance-based methods, have been investigated for virus detection as well. A handheld micro-Raman portable device has been developed for detecting protein-based compounds, focusing on narrow spectral bands encompassing 640–740 cm^{-1}, 1200–1260 cm^{-1}, 1520–1560 cm^{-1}, and 1640–1740 cm^{-1}. NIR spectroscopy has been used for its rapid detection capabilities without the need for reagents or sample preparation (Auner et al., 20122). SERS has demonstrated sensitivity and specificity ranging from 10^4 to 10^9 times higher than conventional Raman spectroscopy (Rusciano et al., 2023). Quantum dots used in fluorescence biosensors offer advantages such as high quantum yield, tunable emission wavelength, photostability, and a significant Stokes shift. However, their application in oxidative environments raises concerns about high cytotoxicity and potential damage to DNA (Pandey and Bodas, 2020). Biocompatible alternatives, like conjugated polymer nanoparticles and carbon dots, have been developed as light-emitting nanomaterials. AI-based deep learning approaches have also been explored for combating COVID-19, including a novel automated screening technique for detecting COVID-19 (Kotta et al., 2020).

5.3.1. Loop-mediated isothermal amplification (LAMP) based assay

Loop-mediated isothermal amplification (LAMP) is a widely recognized method in molecular biology for its rapid and effective pathogen detection capabilities. Introduced in 2000, LAMP has gained popularity due to its simplicity, sensitivity, and specificity (Wong et al., 2018). Recent advancements in LAMP technology include improvements in primer design algorithms, multiplexing LAMP assays, and integration with portable and point-of-care devices. Research has focused on enhancing the sensitivity and specificity of LAMP assays, with modifications to reaction conditions, primer sequences, and the incorporation of loop primers contributing to these improvements (Garg et al., 2022). LAMP has expanded its applications beyond clinical diagnostics to include the detection of pathogens in environmental samples, such as water and soil, for monitoring and controlling the spread of infectious agents. Digital LAMP techniques, like digital PCR, have been developed to quantify target nucleic acids, providing a more accurate assessment of pathogen load. Additionally, LAMP is increasingly used in agriculture for detecting plant pathogens, with efforts have been made to standardize LAMP protocols for better reproducibility and comparability across different laboratories.

5.3.2. Paper-based lateral flow assay

The Lateral Flow Immunoassay (LFIA) is a commercially successful paper-based POC diagnostic device, which has evolved from home pregnancy tests to quickly detect various biomarkers and substances (Sadeghi et al., 2021). LFIA operates on the principle of specific binding between antibodies and antigens, proteins, or hormones. Unlike traditional PCR, LFIA is amplification-free, making it more user-friendly. A typical LFIA device consists of a sample pad, a conjugation pad, and an absorbent pad. The target analyte solution is absorbed in the sample pad and propelled towards the conjugation pad through a cellulose membrane. In the conjugation pad, a labeled antibody forms a conjugate with the analyte and is transported along the membrane. Test lines contain immobilized affinity ligands specific to the target analyte/conjugated antibody complex. The solution continues to flow across the membrane until it reaches the control line, confirming proper assay functionality. LFIA has been used to develop a paper-based POC device for the visual detection of SARS-CoV-2 antibodies in human blood samples (Jia et al., 2021).

5.3.3. Surface plasmon resonance-based assay

Surface Plasmon Resonance (SPR) techniques are increasingly used for the rapid detection of the SARS-CoV-2. These biosensors offer a combination of speed, accuracy, and portability, making them ideal tools for combating the pandemic. (Akib et al., 2021) introduced a highly sensitive SPR biosensor equipped with a graphene-based multiple-layer coating, employing total internal reflection to observe real-time immobilization of ligand-analyte interactions in the detection area. The sensor's performance was assessed using three different analytes and ligands: virus anti-spike proteins, COVID-19 virus spike RBD, monoclonal antibodies, and viral single-stranded ribonucleic acid (RNA) (Fendi et al., 2023). Among the methods, the total virus spike RBD detection showed the greatest sensitivity. To further enhance sensitivity and plasmonic features, highly sensitive two-dimensional (2D) nanomaterials were utilized in conjunction with each other. The dual-functional plasmonic biosensor, which combines the plasmonic photothermal effect with localized surface plasmon resonance (LSPR) sensing transduction, presents a promising alternative for clinical COVID-19 diagnosis. Utilizing two-dimensional gold nano-islands functionalized with complementary DNA receptors, this biosensor facilitates the sensitive detection of specific virus sequences through nucleic acid hybridization (Trzaskowski et al., 2023).

To enhance sensing performance, the biosensor generates thermoplasmonic heat on the same AuNIs chip when illuminated at their plasmonic resonance frequency. This localized PPT heat increases the in-situ hybridization temperature, aiding in the accurate discrimination of two closely related gene sequences. The dual-functional LSPR biosensor exhibits high sensitivity to the selected SARS-CoV-2 sequences, boasting a lower detection limit of 0.22 pM and precise detection of the specific target in a multigene mixture (Qiu et al., 2020). Plasmonic biosensing is a promising method for coronavirus detection, offering rapid testing and reduced manpower requirements. Studies have shown that commercially available SPR and LSPR sensors are effective in detecting viral strains like SARS, MERS, and influenza. Later, Moitra et al. (2022) reported a study on COVID-19 detection using plasmonic nanoparticles, yielding results within 10 min. Ahmadivand et al. (2021) presented a

toroidal plasmonic metasensor for femto-molar detection of the COVID-19 spike protein, with a claimed LOD approximately 4.2 fmol and a sample-to-result duration of about 80 min. Das et al. (2020) designed a gold nanorod-based plasmonic sensor for COVID-19 detection, demonstrating a sensitivity of 111.11 deg/RIU. Ahmed et al. (2018) introduced a sensor utilizing magneto-plasmonic nanoparticles for coronavirus detection, with a LOD of 79.15 EID/50 mL. Huang et al. (2022) developed a localized surface plasmon-coupled fluorescence fibre-optic biosensor for SARS-CoV detection.

5.3.4. Proximity ligation assay (PLA)

Researchers have developed a rapid and accurate quantitative assay for IBV diagnosis using highly specific single-stranded DNA aptamers, which eliminates the need for nucleic acid extraction. This novel assay demonstrated excellent sensitivity and specificity in identifying IBV in farm samples. Compared to the gold standard method, PLA, the newly created sandwich ELAA (enzyme-linked aptamer assay) and qRT-PCR also showed promising results, highlighting their potential for effective IBV diagnosis. Hmila et al. (2023) reported the use of two aptamers, AptIBV5 and AptIBV2, to establish homogenous and solid-phase proximity ligation assays (PLAs). The solid-phase PLA showed a lower detection limit and a broader dynamic range than the other two assays. This technique may serve as an alternative assay for IBV diagnosis and potentially be extended to other important animal or human viruses (Marnissi et al., 2021). Aptamer-based tools have been developed to address the issue of IBV detection. Aptamers are promising nano-molecules in medicine due to their specific recognition of target molecules and high affinity to nanomolar or sub-nanomolar ranges. They offer a robust in vitro selection method using selective ligands by exponential enrichment (SELEX), allowing for highly sensitive detection tests. Combining aptamers with PLA for more sensitive detection of IBV is also being investigated (Yang et al., 2023).

6. Recommendation of selecting suitable biosensor-based technique for different poultry farms

Based on review of multiple studies, providing tailored recommendations for the selection of appropriate biosensor-based technique is essential to accommodate specific breeding conditions, poultry species and disease scenarios.

For large-scale poultry farms, DNA biosensors and immunosensors are the preferred options due to their high sensitivity and capacity to process large sample volumes, such as blood or swab samples from affected farms (Mondal et al., 2024; Lau and Botella, 2017). These farms often require high-throughput diagnostic tools to monitor the health of their flocks on a broad scale, and their higher operating budgets can support the associated costs. In addition, regular testing can be efficiently conducted through centralized diagnostic laboratories or on-site installations.

In contrast, for smallholder poultry farmers, the focus should be on cost-effective and user-friendly diagnostic tools that enable quick decision-making in resource-constrained settings. Portable and affordable biosensors, such as lateral flow immunosensors or electrochemical biosensors, are particularly well-suited for such scenarios (Park, 2022; Yuan et al., 2023). Point-of-care devices with minimal technical requirements are particularly advantageous for field conditions, offering simplicity and practicality for end-users.

For outbreak mitigation efforts, the use of highly sensitive biosensors such as DNA biosensors or optical sensors is recommended for detecting of small pathogen loads in early screening situations. Conversely, immunosensors or rapid lateral flow tests are better suited for rapid, large-scale detection at high infection rates, facilitating timely and effective containment measures (Park, 2022).

These tailored recommendations underline the need to align biosensor selection with the unique demands of different poultry farming contexts, optimizing both disease control efforts and resource utilization.

7. Conclusions

Detection of coronaviruses in poultry remains a cornerstone of efforts to control and mitigate their impact on poultry health and productivity. Sensor-based technologies represent a transformative advancement in this field, offering rapid, accurate, and field-deployable diagnostics that surpass traditional methods. This review highlights recent developments in DNA, immunological, and optical biosensors, as well as nanotechnology, underscoring their potential to enhance virus detection accuracy and support the development of targeted vaccines. Incorporating biosensors into diagnostic strategies makes a significant step forward in poultry health management. Future research should focus on combining sensors with advanced data analytics, such as artificial intelligence, to enable real-time disease surveillance and improve diagnostic precision.

CRediT authorship contribution statement

Md Safiul Alam Bhuiyan: Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Suman Das Gupta:** Writing – review & editing, Visualization, Resources, Methodology, Formal analysis. **Juplikely James Silip:** Writing – review & editing, Visualization, Formal analysis. **Saranika Talukder:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Md Hakimul Haque:** Writing – review & editing, Funding acquisition, Formal analysis. **Jade K. Forwood:** Writing – review & editing, Visualization, Supervision. **Subir Sarker:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Informed consent statement

Not applicable.

Fund

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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