

DOI 10.24425/pjvs.2023.145056

*Review paper*

# Application of aptamer-based viral detection in animals

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## Abstract

Viral infections are common causes of diseases in animals and appropriate methods are increasingly being required to detect viral pathogens in animals. In this regard, similar to antigen-antibody interactions, aptamers have high affinity and specificity for their respective target molecules, and can be selected using the Systematic Evolution of Ligands by EXponential enrichment (SELEX) technique. Recently, significant progress has been made in the development of aptamer selection and aptamer-based sensors for viral detection, and here we review some of the recent advances in aptamer-based detection of viral infections in animals. This review will serve as a comprehensive resource for aptamer-based strategies in viral diagnostics.

**Keywords:** aptamer, virus, biosensor, animal

## Introduction

The detection of viral infections is a vital facet of veterinary science. However, although multiple diagnostic methods have been developed for detecting such infections, there is a constant need for more efficient diagnostic tools, with a focus on aspects such as rapid detection, accuracy, affordability, and portability (Storch 2000). Recently, nucleic acid-based diagnostic methods have been a rapidly developing area in biosensing (Prabhakar and Lakhanpal 2020), among which, aptamers have attracted increasing attention, owing to their applicability in a wide range of disci-

plines, including pathogen and toxin diagnostics (Chakraborty et al. 2022).

Diagnosis of viral pathogens during the early stages of infection is essential for prevention and prompt treatment. Current gold standard methods used for the detection of viral infections include nucleic acid testing (NAT) and antigen-antibody-based enzyme-linked immunosorbent assays (ELISAs), whereas other common methods include viral plaque assays, flow cytometry, and hemagglutination assays (D’Cruz et al. 2020). NAT methods are generally amplification-based enzymatic assays that detect viral genetic material using polymerase chain reactions (PCR). However, although

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Table 1. Comparison of the properties of antibodies and aptamers in virus diagnostics.

Properties	Aptamer	Antibody
Material	Oligonucleotides (DNA or RNA)	Proteins
Target	Wide range of targets such as small molecules, peptides, proteins, cells, and viruses	Wide range of targets such as small molecules, peptides, proteins, and the surface proteins of cells and viruses
Size	~20 kDa	~150 kDa
Immunogenicity	Low	High
Development period	3-6 weeks	Months
Manufacturing	Chemical synthesis	Biological manufacturing
Storage	Room temperature	Cold temperature
Shelf life	Unlimited	Limited
Binding affinity	Nanomolar to picomolar range	Nanomolar to picomolar range
Stability	Stable in various environmental conditions	Special conditions are required for handling and storage
Clinical application	Immature	Mature
Specificity	High	High
Chemical modifications	Easy and controllable	Limited and uncontrollable

NAT-based detection is sensitive, the procedures typically entail labor-intensive operation, laboratory-based sample preparation, and complex result interpretation by skilled personnel. Comparatively, immuno-assays are generally rapid, although less sensitive.

In recent years, as one of NAT methods, aptasensors (aptamer-based biosensors (Lou et al. 2022)) have been increasingly used for viral detection, contributing to enhancements in the monitoring of animal health. In this paper, we have reviewed a selection of some recently published articles on viral detection using aptasensors in animals, and outline the most recent application of aptasensors in this field.

### Comparison of aptamers and their properties with those of antibodies in virus diagnostics

Aptamers are RNA or single-stranded DNA (ssDNA) molecules that can non-covalently bind to a broad range of molecular targets (e.g., small molecules, proteins, cells, and tissues) with high affinity and specificity (Lee et al. 2006, Banerjee and Nilsen-Hamilton 2013, Hong and Sooter 2015). The molecular recognition between aptamers and their targets is based on molecular shape complementarities, stacking of aromatic rings, electrostatic or van der Waals interactions, and hydrogen bonding (Reinemann et al. 2009). Aptamers are selected via an *in vitro* process referred to as Systematic Evolution of Ligands by EXponential enrichment (SELEX) (Ellington and Szostak 1990, Robertson and Joyce 1990, Tuerk and Gold 1990), in which the aptamers are selected and characterized from a random

single-stranded nucleic acid library (generally containing  $10^{15}$ ~ $10^{16}$  different sequences) in three main steps: selection, separation, and amplification. The isolated aptamers can be used for various applications, including target detection for virus diagnostics (Wu et al. 2014, Darmostuk et al. 2015, Ouellet et al. 2015, Romero-Lopez and Berzal-Herranz 2017).

Compared with antibody generation, SELEX processes facilitate greater control over binding conditions (Kim and Gu 2014, Pfeiffer and Mayer 2016), and DNA is also characterized by higher stability and tolerance under different physical and chemical conditions (e.g., high temperatures), as compared to antibodies (Iliuk et al. 2011, Jafari et al. 2018). The generation of aptamers for detecting viral infections has recently been well-reviewed by Chakraborty et al. (2022). These single-stranded probes offer several advantages over antibodies, including reusability, stability, and lack of immunogenicity. In addition, their ease of synthesis and modification makes them ideal agents for the recognition of viral infections in most biological samples (Table 1) (Sett et al. 2014).

### Applications of aptasensors for viral detection in animals

Consequently, considerable efforts have been made to develop alternative approaches for the more rapid and economical detection of viral infections in animals. In this regard, different types aptasensors have recently been developed for the detection of animal viruses (Table 2).

Table 2 Aptasensors used in the detection of viruses infecting animals.

Virus	Target	Sensor types	Limit of detection	Chemistry	Length (nt)	Sequence (5'-3)'	Year	Reference
Foot-and-mouth (FMD) virus	VP1 structural polypeptide of Oserotype FMD	FRET (Fluorescence resonance energy transfer)	250 ng/mL 25-250 ng/mL	DNA	72	ATACGGGAGCCAACAC CATTCTATCGTTCCGGAC GCTTATGCCTTGCCATC TACAGAGCAGGTGTGAC GGAT	2008	(Bruno et al. 2008)
Vesicular stomatitis virus (VSV)	Whole virus detection: Vesicular stomatitis virus (VSV)	Electro-chemical	600 PFU	DNA	/	A selected pool	2012	(Labib et al. 2012)
Hirame rhabdovirus (HIRRV)	Whole virus	/	4.76×10 <sup>3</sup> to 2.13×10 <sup>4</sup> PFU mL <sup>-1</sup> (for 0.25 nmol aptamer)	RNA	40/41/38/40	Sequence 1: GGUGUAUAGGU AUUGAAUCAUU GUUUUAGCGUUGUCUUA Sequence 2: GGAACGCGAGCUUA GAUUUCGGAGAA CAGUUUGUAAUUGUU Sequence 3: GUCCUAUACAGUGUGAC CGUUUGAAAGUGGGUUC GUUG Sequence 4: GGUUUAGCCGGAUU GUAGACUGUAAACAGGUA GAGCUGGC	2012	(Hwang et al. 2012)
Bovine viral diarrhea virus type 1 (BVDV type 1)	Whole virus	SPR (Surface plasmon resonance)	800 copies/mL	DNA	67/66	Sequence 1: biotin CGTACGGAATTC GCTAGCTGCGCATCCA CAAATGTATTGTCGGGG GAATGGATCCGAGCTC CACGTG Sequence 2: Thiol CGTACGGAATTC GCTAGCTGCGGACTCGC GATGCTACTTCTGATGA TAGGATCCGAGCTCCAC GTG	2014	(Park et al. 2014)
Singapore grouper iridovirus (SGIV)	SGIV (strain 3/12/98)	ELASA (Enzyme-linked apta-sorbent assay)	5 × 10 <sup>4</sup>	DNA	82	GACGCTTACTCAGGTGT GACTCGTGGCTCTTTAT GTTTTTGGGAGGGTTG GCTCGTATGTCGAAGGAC GCAGATGAAGTCTC	2016	(Li et al. 2016)
Muscovy duck parvovirus (MDPV)	Duck embryo infected with MDPV	AuNP-mediated, label free detection by microtiter plate assay	1.5 or 3 EID50	DNA	49	TAATCGCGTAACGGTGC CGTTGCCATAGAAAAGG GTATCTGGGTCGTGC	2018	(Lu et al. 2018)
Drosophila C virus (DCV)	Whole virus	Enzyme linked chemiluminescence sandwich assay	0.7 µg	DNA	71	CTTACCTCTTTCTCCCAC CGCCATTCTTTTCTAC GCCTTCCATTACGCACAA CACACAACAATCAATA	2018	(Damase et al. 2018)
Nervous necrosis virus (NNV)	Coat protein of red spotted grouper nervous necrosis virus (RGNNV-CP)	LFA (Lateral flow assay)	5 ng/mL	DNA	79/50	Sequence 1: TTCTTTTATTAGTT GATTTTTTTGATTTTGG CAGCTACTGCTTTGGGG GTGCTGAGGCGAAGGAC GCAGATGAAG TCTC Sequence 2: TGTGTGTCTATTGTCT GTGGTTCATGTCAAGCT TATTT TCCACACACGGT	2020	(Liu et al. 2020)

PFU – Plaque formation unit; EID50 – Tissue culture infective dose

## Monitoring of the foot-and-mouth virus

Although there have been no outbreaks of the Foot-and-mouth virus (FMD) in the United States since 1929, outbreaks in Asia and South America have indicated the necessity of early detection to prevent recurrences. In 2008, Bruno et al (2008), developed a novel competitive fluorescence resonance energy transfer aptasensor (FRET) for the rapid detection of FMD within 10 min. FRET is a nonradiative process of energy transfer based on the dipole-dipole interactions between molecules that are fluorescent. Transfer of energy takes place rapidly from a donor molecule to an acceptor molecule in juxtaposition such as 0 to 10 nm without photonic radiation. FRET has occupied a center stage in biotechnology and biological studies (Kaur et al. 2021). In this system, FMD polyclonal DNA aptamers are labeled with Alexa Fluor 546-14-dUTP (which was used to synthesize labeled DNA probes with fluorescence) and allowed to bind the BHQ-2-peptide conjugate (Black Hole Quencher 2 conjugated with FMD peptides) to detecting the FMD. Results showed that FMD peptides can be detected in the range of between 25 and 250 ng/mL.

## Monitoring of the vesicular stomatitis virus

The vesicular stomatitis virus (VSV) is a negative-stranded RNA virus that primarily infects rodents, cattle, pigs, and horses. Although this virus has been widely used in cell physiology studies, its potential application as a cancer therapeutic has received increasing attention only in the past decade (Lichty et al. 2004). Recently, an electrochemical aptasensor for the quantification of VSV was developed via self-assembling of a thiolated ssDNA primer and VSV-specific aptamer (Labib et al. 2012). In this system, the thiolated ssDNA primer hybridized with VSV-specific aptamer was coated on the response surface, once VSV binding with aptamer can induce the release of aptamer from the surface contributing to the signal change. Thus, VSV can be detect correctly in the range of 800 to 2200 plaque-forming units (PFU), with a detection limit of 600 PFU.

## Monitoring of the Singapore grouper iridovirus

The Singapore grouper iridovirus (SGIV) is a destructive aquaculture-associated virus that causes significant economic damage to groupers. In 2016, Li et al. (2016) developed a novel aptamer-based enzyme-linked apta-sorbent assay (ELASA) for the detection of SGIV

infection in the grouper *Epinephelus coioides*. Biotin-labeled aptamers, streptavidin-horseradish peroxidase (SA-HRP), and 3,3', 5,5-tetramethylbenzidine (TMB) chromogen solution were used in the sensing system. This aptasensor enables the rapid detection of SGIV infection with high specificity and stability, both in vitro and in vivo. SGIV particles can be detected at concentrations as low as 125 nmol, and in vivo, it can be detected in kidney, liver, and spleen samples at dilutions of 1/50, 1/100, and 1/50, respectively.

## Monitoring of the muscovy duck parvovirus

The muscovy duck parvovirus (MDPV) is associated with high mortality and morbidity in ducks. Recently, by using unmodified Au nanoparticles (AuNPs) and aptamers, a aptasensor was established for monitoring MDPV with a detection limit of 1.5 EID<sub>50</sub> (50% egg infection dose). Reactions can also be observed with the naked eye at a detection limit of 3 EID<sub>50</sub> (Lu et al. 2018). These findings accordingly demonstrate the application potential of aptamers in the rapid detection and monitoring of MDPV infection as well as antiviral research.

## Drosophila C virus

Drosophila C virus (DCV) is a positive-sense RNA virus belonging to the Dicistroviridae family affecting the widely-studied model organism *Drosophila melanogaster*. Damase et al. (2018) developed a aptamer sandwich luminescence assay for the specific and rapid detection of DCV with a limit of detection (LOD) of 0.7 µg of virus. The aptamer conjugated microspheres, DCV and biotinylated aptamer were used to build the sandwich luminescence detection system. Moreover, when using this assay, there is no cross-reaction with the *Drosophila X* virus, thereby highlighting the specificity of this assay.

## Nervous necrosis virus (NNV)

To date, nervous necrosis virus (NNV) infections have been detected in more than 50 species of fish worldwide, causing substantial economic losses to the aquaculture industry. There is an urgent need to develop a rapid and accurate method for the prevention and control of NNV infections. In 2020, a simple and sensitive aptamer-based lateral flow biosensor was developed for the rapid detection of red-spotted grouper nervous necrosis virus (RGNNV) (Liu et al. 2020), which can detect RGNNV-CP protein levels as low as 5 ng/mL

or  $5 \times 10^3$  RGNNV-infected grouper brain cells. Two modified aptamers were used in this study. One aptamer was used for magnetic bead enrichment and the other was used for isothermal strand displacement amplification (SDA). After amplification, the product was further tested by the LFB, and the detection results were observed by the naked eye within 5 min with high specificity and sensitivity.

### **Applications of aptasensors for the detection of viruses infecting both animals and humans**

Considerable efforts have been made to develop different types aptasensors for the detection of viruses infecting both animals and humans (Table 3).

#### **Monitoring of the influenza virus**

##### **Monitoring of H5N1**

For more than a decade, the H5N1 subtype of the Avian influenza virus (AIV) has caused the most lethal outbreaks of highly pathogenic avian influenza in poultry and also fatal infections in humans. H5N1 infections have caused substantial economic losses in countries and regions worldwide, and also the virus has the ability to mutate or recombine with other subtypes, thereby becoming a lethal pathogen that constantly poses a threat to human health (Lum et al. 2015).

Considerable efforts have been made worldwide to develop a system for the rapid detection and monitoring of H5N1 viruses. For example, Bai et al. (2012) developed a surface plasmon resonance (SPR)-based portable aptasensor for the rapid detection of H5N1 in poultry swab samples. SPR is a sensitive surface analysis technique that detects changes in the dielectric constant caused by molecular adsorption on heavy metal films. This aptasensor enabled the detection of H5N1 in 1.5 h with an LOD of 0.128 hemagglutinin units (HAU). Lum et al. (2015) established a microfluidic chip-based impedance aptasensor with a detection time of 30 min and detection limit of 0.0128 HAU. The biotinylated aptamer/streptavidin modified microelectrode surface was constructed in the microfluidic channels. The target virus was captured on the microelectrode surface, causing an increase in the impedance magnitude. In addition, other groups have developed aptasensors that can be used to detect H5N1 within 30 min to 1 h with detection limits of  $2 \times 10^{-9}$  HAU (Wang et al. 2015), 100 fM (Diba et al. 2015), and 0.0128 HAU (Wang and Li 2013, Hmila et al. 2017). Moreover, these aptasensors are specific to H5N1 and

do not cross-react with other AIV subtypes (e.g., H1N1, H2N2, and H7N2). They can also be used for detection in diluted human serum samples spiked with different concentrations of target viral protein.

##### **Monitoring of H3N2**

Chen et al. (2016) developed a magnetic bead-based colorimetric platform for H3N2 with an LOD of 11.16 g/mL. The sensor consists of AuNPs modified with concanavalin A and glucose oxidase (ConA-GOx-AuNPs). This platform was developed by using the high efficiency of enzymatic catalysis and the reduction of gold ions with hydrogen peroxide.

##### **Monitoring of H9N2**

Le et al. (2014) developed an aptasensor for the detection of H9N2 that can be used to detect viral particles at levels down to  $3 \times 10^8$ , in both buffers and stimulated nasal mucus, with reactions being visible to the naked eye. This detection level corresponds to the upper end of a typical viral load in 1 mL of human respiratory specimens.

##### **Monitoring of coronavirus disease 2019**

Among the detection systems developed to date is a colorimetric sandwich aptasensor that can detect pseudo-typed lentiviruses in 50% saliva with an LOD of 400 fM, thereby confirming the potential of aptasensors as a diagnostic tool for Corona Virus Disease 2019 (COVID-19) detection (Li et al. 2021). In addition, Chen et al. (2020) have reported that using an enzyme-linked aptamer assay (ELAA), the SARS-CoV-2 N protein can be detected at concentrations as low as 10 ng/mL, and a further aptasensor developed by Kacherovsky et al. (2021) can be used to detect UV-inactivated SARS-CoV-2 at concentrations as low as  $5 \times 10^5$  copies/mL.

Furthermore, Zhang et al. (2021) have developed an aptasensor capable of detecting 1000 viral particles per mL in 1:1 diluted saliva in less than 10 min, and without the necessity of additional sample processing. When used to evaluate the saliva samples of 36 positive and 37 negative patients, this system was shown to achieve a clinical sensitivity of 80.5% and specificity of 100%, with the sensor detecting the wild-type virus as well as alpha and delta variants in the patient samples (Zhang et al. 2021).

##### **Monitoring of bovine viral diarrhea virus type 1**

Bovine viral diarrhea virus (BVDV) is an umbrella term for two species of virus, BVDV1 and BVDV2,

Table 3. Viruses infecting humans and animals.

Class	Target	Sensor types	Limit of detection	Chemistry	Length (nt)	Sequence (5'-3')	Year	Reference
Influenza virus H5N1	Hemeagglutinin protein (HA)	SPR (Surface plasmon resonance)	0.128 HAU	DNA	73	GTGTGCATGGATAGCACGTAACGGTGTAG TAGATACGTGCGGGTAGGAAGAAAGG GAAATAGTTGTCTCTGTTG	2012	(Bai et al. 2012)
Influenza virus H5N1	Hemeagglutinin protein (HA)	Impedance	0.0128 HAU	DNA	73	GTG TGC ATG GAT AGC ACG TAA CGG TGT AGT AGA TAC GTG CGG GTA GGA AGA AAG GGA AAT AGT TGT CCT GTT G	2015	(Lum et al. 2015)
Influenza virus H5N1	Hemeagglutinin protein (HA)	Electrochemical	2 <sup>-9</sup> HAU	DNA	73	GTGTGCATGGATAGCACGTAACGGTGTAG TAGATACGTGCGGGTAGGAAGAAAGG GAAATAGTTGTCTCTGTTG	2015	(Wang et al. 2015)
Influenza virus H5N1	Hemeagglutinin protein (HA)	Electrochemical	100 fM	DNA	28	TTGGGGTTATTTTGGGAGGGCGGGGGTT	2015	(Diba et al. 2015)
Influenza virus H5N1	Hemeagglutinin protein (HA)	QCM (Quartz crystal microbalance)	0.0128 HAU	DNA	73	GTGTGCATGGATAGCACGTAACGGTGTAG TAGTAACGTGCGGGTAGGAAGAAAGG GAAATAGTTGTCTCTGTTG	2013	(Wang and Li 2013)
Influenza virus H3N2	Surface protein	Colorimetry	11.16 µg/mL	DNA	67	NH2- AATTAACCCTCACTAAAGGGCT GAGTCTCAAAAACCGCAATAACTGGTTG TATGGTCAATAAGTTAA	2016	(Chen et al. 2016)
Influenza virus H9N2	/	Immuno-PCR	100 TCID50/mL	DNA	40	CCTTGTCTATTGAACCTCTTAGTCTG GTCTCAGTTG GG	2017	(Hmila et al. 2017)
Influenza virus H9N2	H1N1, H3N2, and influenza B)	Fluorescence readout using microfluidic system	3.2 HAU	DNA	72	ACAGCACCACAGACCACCCGCGGATGC CGGTCCCTA CGCGTCGCTGTCACGCTGGCT GTTTGTCTTCTGCGC	2016	(Chen et al. 2016)
Influenza virus H9N2	Influenza B Virus B/Johannesburg/05/1999 (J1999V)	Gold nanoparticle detection	3 × 10 <sup>8</sup> viral particles	RNA	88	GGGAGCUCAGCCUUCACUGCACUCCG GCUGGUGGA CGCGGUACGAGCAUUU GUACCGGAUGGAUGUUCG GGCAGCGGU CGGAUCCAC	2014-2012	(Negri et al. 2012, Le et al. 2014)
SARS-CoV-2	Nucleocapsid (N) protein	Direct ELAA (enzyme-linked aptamer assay) in buffer	0.01 µg	DNA	88	biotin GCAATGGTACGGTACTTCCGGATGCG GAAACTGGCT AATTGGTGAGGCTGGG GCGGTCGTGCAGCAAAAGTG CACGC TACTTGTCTAA	2020	(Chen et al. 2020)
SARS-CoV-2	Nucleocapsid (N) protein	Direct ELAA in buffer	0.01 µg	DNA	78	biotinGCAATGGTACGGTACTTCCGGATGCG GGAAACTGGCT AATTGGT GAGGCTGGGGCGGTCTGCAGCAAAAGT GCACGCT 78nt	2020	(Chen et al. 2020)
SARS-CoV-2	Nucleocapsid (N) protein	Direct ELAA in buffer	0.01 µg	DNA	57	biotinGCAATGGTACGGTACTTCCGGATGCG GGAAACTGGCT AATTGGT GAGGCTGGGGCGGT 57nt	2020	(Chen et al. 2020)
SARS-CoV-2	Spike (S) protein	Colorimetric sandwich assay	400 fM pseudo-typed virus	DNA	79	TTACGTCAAGGTGTCACCTCCACTTCCG GTTAATTTAIGCTCTACCCGTCCACCTAC CGGAAGCATCTCTTTGGCGTG	2021	(Li et al. 2021)
SARS-CoV-2	Spike (S) protein	LFA (Lateral flow assay) or ELISA (enzyme-linked immunosorbent assay)	2.5×10 <sup>7</sup> or 5×10 <sup>5</sup> copies/mL	DNA	86	TCGCTCTTCCGCTTCTTCGCGGT CATTGTGCATCCT GACTGACCCTAAGGT GCGAACATCGCCCGGTAAAGT CCGTGT GTGCGAA	2021	(Kacheroovsky et al. 2021)
SARS-CoV-2	Spike (S) protein	EIS (Electrochemical impedance sensor)	1×10 <sup>3</sup> copies/mL	DNA	108	TTCCGGTTAATTTATGCTCTACCCGTC CACCTACCGGAATTTTTTTTTTTTTTTTT TTTTTTTTTTACGGGTTTG GCGTCGGG CTGGCGGGGGATAGTGCAGT	2021	(Zhang et al. 2021)
Bovine viral diarrhoea virus type 1	Whole virus particle	Aptamer-aptamer sandwich type sensing	500 TCID50/mL or 800 copies/mL	DNA	66	CGTACGGAATTCGCTAGCTGCGCATC CACAAATGTAT TGTCGGGGATGGATC CGAGCTCCACGTG	2014	(Park et al. 2014)
Rift Valley fever virus (RVFV)	N protein	Fluorescence polarization	Kd=2.6 µM	RNA	9	AAAGACCCC	2012	(Ellenbecker et al. 2012)
Enterovirus	Enteroviral RNA sequence	Colorimetry, spectroscopy and LFA (Lateral flow assay)	100 nM	DNA	20	GAAAC ACG GAC ACC CAA AGT	2021	(Chauhan et al. 2021)
West Nile fever virus (WNV)	E gene	Liquid array detection	10 copies	DNA	25	TGAAGGGAACAACCTATGGCGTCTG	2018	(Wang et al. 2018)

HAU – hemagglutination unit, EID50 – Tissue culture infective dose, Kd – Dissociation constant

within the genus *Pestivirus* of the *Flavivirus* family. The advantage of using of aptamers generated from cell-based selections was that they can target to the whole cell as well as the whole virus. Park et al. (2014) developed a sandwich aptasensor for detection of the whole BVDV1 virus, for which an LOD of  $5 \times 10^2$  TCID<sub>50</sub>/mL was obtained, which is estimated to be equivalent to 800 copies/mL, similar to that obtained using real-time PCR-based detection.

### Monitoring of enteroviruses

Enteroviruses are ubiquitous mammalian pathogens that can cause mild-to-life-threatening diseases upon infection. Chauhan et al. (2021) developed an aptasensor for the rapid detection of enteroviruses, the aptamers of which were designed to achieve specific complementarity with the target enteroviral RNA, thereby forming aggregated gold-aptamer nano-constructs. The conserved target enteroviral nucleic acid sequence initiates gold aptamer-nano-construct disaggregation and a signal transduction mechanism, thereby resulting in a colorimetric and spectroscopic blueshift. Furthermore, an aptasensor that utilize gold-aptamer-nano-constructs has been demonstrated to be unaffected by contaminating human genomic DNA, with rapid detection of conserved target enteroviral nucleic acid sequences (< 60 s), and results that can be interpreted using a bespoke software and hardware electronic interface.

### Conclusion and perspectives

Recently, considerable efforts have been made to realize the goal of high-performance detection of viral infections in animals using different types of biosensors. Although antibodies show high specificity towards their respective antigens, certain features of some proteins, e.g. low temperature requirements for storage and transportation and short shelf life, considerably limit their utility. Thus, on the basis of the developments summarized in this review, we conclude that nucleic acid-based biosensors (e.g., aptamers, functional nucleic acids (FNA), and peptide nucleic acids (PNA)) are the main systems on which to base further sensor development. Although the developments of aptasensor detection systems continues to be the most prominent, there are certain fields of application for which aptasensors or other sensors for viral infections in animals still require further development. The following are some suggestions for the future application of aptasensors.

(1) The generation of aptamers with higher affinity for viral antigens based on modification of the SELEX process.

- (2) The attainment of highly sensitive and selective detection and analysis of targets based on the development of more powerful signal amplification methods.
- (3) Ultra-fast, simple, label-free, cost-effective, multiplex analyses of complex animal samples.
- (4) Portable analyses based on the integration of sensor elements.

An important factor to be considered in all such developments is that animal physiological samples are considerably more complex than solutions, such as selection buffers, used in the laboratory. Such samples frequently contain numerous molecules that can potentially contribute to signal interference, which may cause non-specific interactions with aptamers or false binding to the target molecules. In the future, affordable, practical, rapid, sensitive, and efficient aptasensors and other detection methods should be developed to overcome the current limitations associated with the detection of viral infection in unspiked animal samples. In addition, it is worth focusing on the isolation and removal of viral infections in animals using aptamer-based or other methods.

### Acknowledgements

This work was supported by the National Key R&D Program of China (2021YFF0703300) and the Educational Commission of the Guangdong Province of China (2022KTSCX284).

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