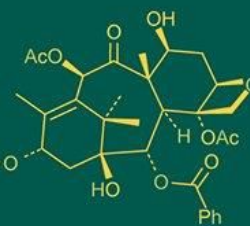


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(1): 274-280
www.biochemjournal.com
Received: 24-10-2024
Accepted: 26-11-2024

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A brief review on Aptamer-based disease diagnosis in the livestock sector

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DOI: <https://doi.org/10.33545/26174693.2025.v9.i1Sd.3480>

Abstract

Aptamers are typically 22–100 nucleobases long and comprise a variable region surrounded by constant regions. Amplification and identification of sequences are made possible by aptamers, which are single-stranded nucleic acids (DNA, RNA) with molecular recognition properties similar to antibodies (Gu, 2016; Kanwar, Roy and Kanwar, 2011; Simmons *et al.*, 2012). Similar to monoclonal antibodies, aptamers have a variety of uses, including the discovery of biomarkers, treatments, diagnostics, and high throughput screening (Shigdar *et al.*, 2011). The ability of the aptamers in detection of the circulating biomarkers can be efficiently done by using ELA (Enzyme Linked Aptamer) assay in targeted drug discovery (de Araujo *et al.*, 2015).

Keywords: ELA (Enzyme Linked Aptamer), SELEX, infectious, synthetic antibodies, FMD

Introduction

1. Aptamers synthesis technique

Aptamers are suitable substitutes for antibodies since they can be chemically synthesised and exhibit specificity equivalent to antibodies for antigen capture and identification (Thivyanathan and Gorenstein, 2012) ^[58]. As a result, they are sometimes referred to as "synthetic antibodies" and have been in direct competition with antibodies in the study of disease detection, prevention, and treatment. They have benefits over antibodies, including lower price, smaller size, simplicity of manufacture and customization, and better stability (Song *et al.*, 2008) ^[53]. In certain scenarios, aptamers are considered a better analog to monoclonal antibodies with respect to higher specificity, low immunogenicity and for simple in vitro selection (Niederlender, Fontaine and Karadjian, 2021) ^[41]. As a result, several aptamer-based systems in the form of biosensors (referred to as apta-sensors) have been created for the detection of pathogens and target analytes, immunophenotyping, and management of animal health. Accordingly apta-sensors also focus on the 'One Health' approach which benefits breeders, animal owners as well as the animals from both infectious and non-infectious diseases.

Tuerk and Gold (1990) ^[59] provided a description of the traditional SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technique. This approach produces aptamers that can last anywhere from a few weeks to a month. By using the same chemical process used to select aptamers and often used to hybridise primers during polymerase chain reaction (PCR), a library of oligonucleotides with distinctive but random sequences was created. The kind of the required aptamer is the only factor that influences several of the selection processes established during the traditional approach of SELEX (Fig. 1). Nucleic-acid aptamers are receiving a lot of attention and have been used in a variety of contexts. The use of aptamers in the identification of human illnesses has been extensively studied; however there is very little information accessible in the field of veterinary medicine. As a result, the emphasis of this study will be on the use of aptamers in the diagnosis of animal illnesses, the detection of drug residues and pollutants, and the administration of targeted medication.

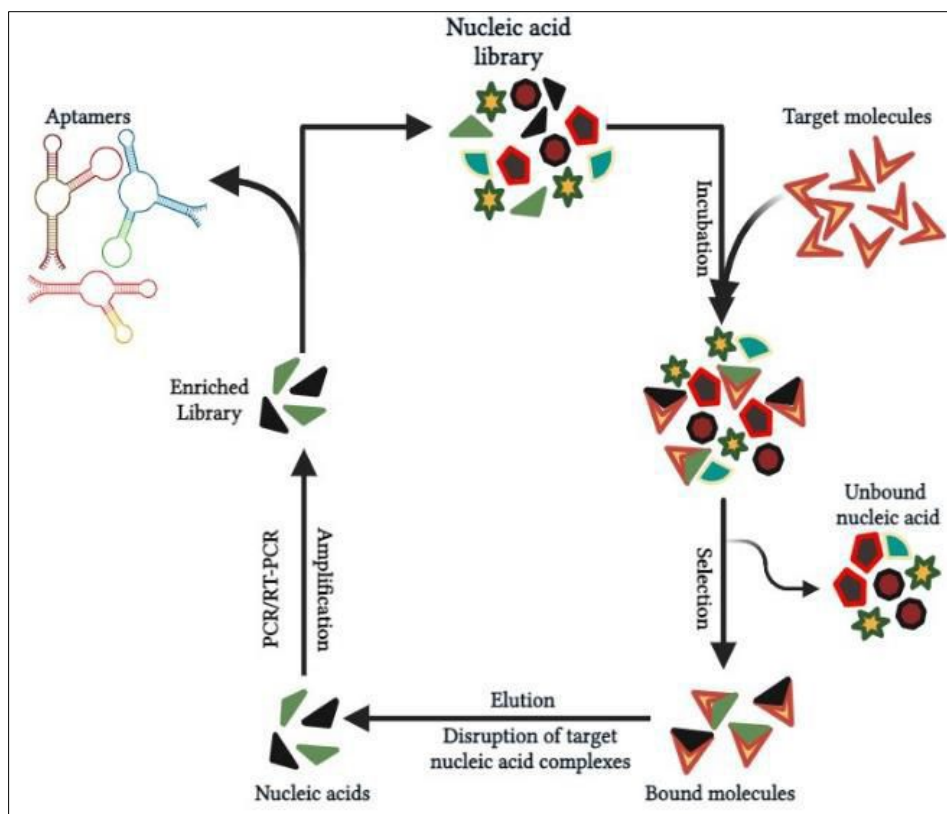


Fig 1: Schematic showing Systematic evolution of ligands by exponential enrichment (SELEX) for the selection of aptamers. (Created using BioRender)

There are various bacterial (e.g. mastitis, tuberculosis, enteritis or colibacillosis, tuberculosis etc.) and viral diseases (e. g. SARS CoV, influenza, FMD etc.) that have a high prevalence rate in animals and are affecting farmers' economies in many ways (Fig. 2). Other conditions, such as bovine spongiform encephalopathy (BSE), cancer, and toxins, play important roles in veterinary medicine in addition to infectious diseases.

2. Diagnosis of viral diseases

2.1 Influenza

Several aptamers have been reported to be effective in several viruses such as Muscovy duck parvovirus (Lu *et al.*, 2018), highly pathogenic avian influenza (HPAI) A(H5) virus (Nguyen *et al.*, 2017)^[40], H5N1 avian influenza virus (Wu *et al.*, 2008), Triple reassortant influenza A virus (Wongphatcharachai *et al.*, 2013)^[64] etc.

2.2 Severe acute respiratory syndrome corona virus (SARS-CoV)

The recent pandemics and epizootics in various parts of the world have highlighted the public health importance of viruses such COVID-19 (SARS-CoV-2), influenza virus, herpes viruses etc. At the beginning of January 2020, a novel coronavirus (COVID-19) was identified in Wuhan, China as a causal agent of an outbreak of respiratory disease. SARS-CoV-2 (Severe Acute Respiratory Syndrome- Coronavirus-2) is a member of Coronaviridae, which is the responsible agent for COVID-19. SARS-CoV-2 is an enveloped virus measuring approximately 50–200 nm in diameter, having single strand RNA genome consists of 26–32 kb in length (Lu *et al.*, 2020; Xu *et al.*, 2020). WHO declared this disease a pandemic on 11 March 2020. COVID-19 has presently spread in the 222 countries/territories with the death of 3.27 million out of

total positive cases, which accounts for more than 156.75 million upto 07 May 2021. While in India alone, 2.15 million are infected and 0.234 million deaths have been reported.

2.3 Foot and mouth disease (FMD)

FMD mainly affects the animals such as swine, cattle, goats, deer, and sheep. FMD affects extensive areas worldwide enlisted in the notifiable diseases to the World Organization for Animal Health. Since it was recognized as a significant epidemic disease in 16th century to yet it is a major global animal health problem (Longjam *et al.*, 2011)^[33]. FMD has caused numerous outbreaks in the US, UK and Asia including India. Owing to significant economic losses there is an urgent need to develop a fast and most unswerving test for the detection of FMD virus. In order to detect this disease, DNA aptamers were successfully screened (Vidic *et al.*, 2017)^[61]. Ellingham *et al.*, 2006^[13] reported the use of anti-3D pol aptamers that successfully inhibited the activity of RNA dependent RNA polymerase from FMD.

2.4 Rabies

Rabies is considered to be one of the most deadly viral infectious zoonotic diseases affecting CNS and becomes 100% fatal when the symptoms begin to appear, as reported by WHO. The causative agent responsible for the transmission of rabies from infected dogs to humans is an unsegmented negative sense RNA rabies virus (RABV). It belongs to the Lyssavirus of the Rhabdoviridae family. Both humans and animals are the victims of this virus mainly propagated via saliva or through direct contact with mucosa mainly open wounds, mouth, and eyes. According to WHO reports in 2023, India accounts for almost 36% of the world's deaths due to the rabies virus. The frequent victims of the RABV are children between the ages of 5 and 14

years. Liang *et al.*, 2014^[31] successfully generated a ssDNA, GE54 aptamer against the RABV cultured in baby hamster kidney (BHK)-21 cells. The GE54 aptamer significantly protected healthy cells from the replication of

RABV both in vitro and in vivo. Another class of DNA aptamers, the F021 and F024 acted on RABV most effectively than GE54 due to their ability of binding with more number of targeted molecules (Liang *et al.*, 2014)^[31].

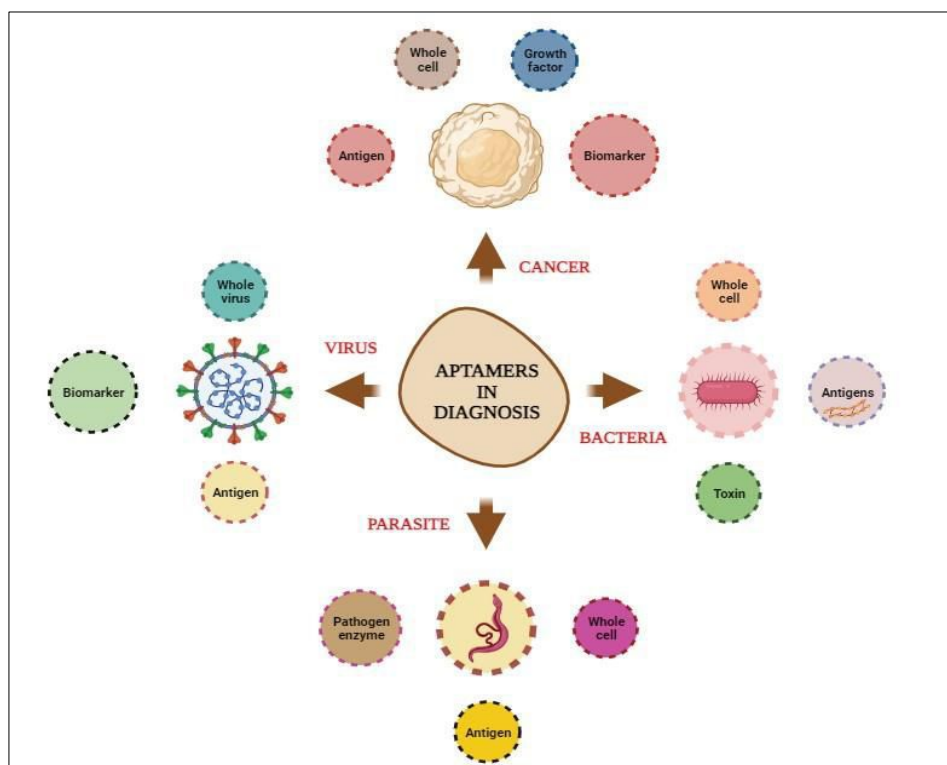


Fig 2: Role of aptamers in diagnosis of cancer and other viral, bacterial and parasitic diseases. (Created using BioRender)

3. Diagnosis of bacterial diseases

3.1 Mastitis

Bovine mastitis can be well-defined as ‘inflammation of mammary gland’ and is one of the most common and deadly diseases of dairy cows and poses substantial economic loss to dairy industry. The estimated annual economic loss due to both subclinical and clinical mastitis in India was US\$ 98,228 million [7165.51 crore Indian Rupees] (Bansal and Gupta, 2009). It is among the most critical diseases of cattle and buffalo because it causes numerous complications to milk production, processing of milk and quality of milk and its products, resulting in severe economic losses to the dairy industry. Mastitis is caused by more than 200 infectious microorganisms and commonest pathogens are *Staphylococcus aureus*, *E. coli*, *Streptococcus* spp., *Pseudomonas* spp., *Mycoplasma* spp. etc. (Sharma and Maiti, 2010)^[46]. Cheng *et al.* (2016)^[7] developed aptamer-coated magnetic beads and antibiotic-capped gold nanoclusters for the detection of bacteria. Baumstummiller *et al.*, 2014^[2] identified SOMamer (slow off-rate modified aptamer) to detect *S. aureus*. SOMamers are made from ssDNA that contain pyrimidine residues and have quite long (>30 min) dissociation rates (Gold *et al.*, 2010). They found that SpA and Clf ASOMamers are useful for the selective detection of *S. aureus*. Another study used ELONA (Enzyme-Linked Oligo Nucleotide Assay) to detect the Protein A-binding aptamer PA#2/8 in *S. aureus* (Stoltenburg *et al.*, 2016)^[56].

3.2 Tuberculosis

Bovine tuberculosis (BT) is a slow progressing disease and primarily caused by the *Mycobacterium bovis* (*M. bovis*),

and to a lesser extent by *M. caprae* and *M. tuberculosis*. *M. bovis* also causes arthritis, mastitis and respiratory diseases in cattle. Serological detection of *M. bovis* has been a concern due to compromised conventional approach. Fu *et al.*, 2014 designed a single stranded-DNA (ssDNA) aptamer WKB-14 that bound specifically to *M. bovis* in a competitive indirect enzyme linked aptamer assay (ELAA). Bovine tuberculosis is affecting the livelihoods and is hampering efforts to control and/ or elimination by 2030 (Cousins, 2018)^[8]. The traditional diagnostic test i.e. tuberculin skin test, is a standard and the most popular test in the livestock. However, the bacterial culture of *M. bovis* is a time-consuming process and can take eight weeks or more. Aptamers are hopeful tools for developing POC diagnostic assays for TB. Mozioglu *et al.*, 2016^[38] developed ssDNA aptamers that recognize *M. tuberculosis* H37Ra using SELEX procedure. Bovine tuberculosis caused by *M. tuberculosis* is difficult to be identified by the pathological symptoms. Release of bovine interferon gamma (BoIFN- γ) by T-cells can offer a diagnostic marker of *M. tuberculosis* (Crulhas *et al.*, 2017)^[9]. Li *et al.*, 2018 reported a voltammetric apta-sensor for detecting ESAT-6, a secretory antigen secreted by *Mycobacterium Tuberculosis*.

3.3 *E. coli*

Escherichia coli is ubiquitously found bacterial species in the intestinal gut flora of animals and can survive in multiple abiotic environments (Marton *et al.*, 2016)^[36]. *E. coli* benefits the host after colonizing the infant's gastrointestinal tract. Still, it may become a potent disease-causing pathogen due to its virulence factors and ability to adapt to different niches causing high mortality (Kaper,

Nataro, Mobley, 2004)^[26]. Based on the type of disease intestinal pathogenic *E. coli* can be classified into entero-hemorrhagic (EHEC), diffusely adherent (DAEC), entero-

pathogenic (EPEC), and entero-invasive *E. coli* (EIEC). Various aptamers were reported to bind against specific *E. coli* species and are tabulated below:

Table 1: Summary of aptamers for *E. coli* diagnosis

S. No	<i>E. coli</i> species	Aptamer	Reference
1.	<i>E. coli</i> O157:H7	A1/AuNP-anti-Ecoli O157:H7 aptamer	Wu <i>et al.</i> , 2012
2.	<i>Enter Toxigenic E. coli</i> (ETEC) K88	Aptamer B12	Peng <i>et al.</i> , 2014 ^[44]
3.	<i>E. coli</i> NSM59	EcA5-27	Savory <i>et al.</i> , 2014 ^[45]
4.	<i>E. coli</i> KCTC 2571	E2 SSDNA aptamer	Kim <i>et al.</i> , 2013 ^[28]
5.	<i>E. coli</i> ATCC 25922	P12-55 SSDNA aptamer	Marton <i>et al.</i> , 2016 ^[36]
6.	<i>E. coli</i> O157:H7	E1	Zhang <i>et al.</i> , 2019 ^[70]
7.	<i>E. coli</i> O157:H7	E18R SSDNA aptamer	Siddiqui and Yuan, 2021 ^[49]

Wei *et al.*, 2018 reported a user-friendly multifunctional spin chip that allows quantitative detection of *E. coli* using nanoparticle-based aptamer recognition. Aptamer binding Microchip capillary electrophoresis method was used for the detection of *E. coli* O157:H7 by coupling with laser-induced fluorescence (Zhang *et al.*, 2019)^[70].

4. Diagnosis of Parasitic diseases

Protozoan parasite infections are a major global health issue, particularly in developing countries, due to poor sanitation and weak immune systems. Current diagnostic methods, like microscopic observation, immune detection, and PCR, are effective but limited by their time-consuming nature, need for cryogenic storage, and specialized equipment, which are scarce in remote areas. Research is now focusing on new diagnostic targets, with aptamers emerging as a promising tool. Aptamers are the synthetic molecules that bind specifically to targets, offer advantages such as stability and ease of production, potentially overcoming the limitations of existing diagnostic techniques. Researchers have expressed significant concern regarding the application of aptamers in the detection of parasitic diseases. Initially, a sandwich model of aptasensors (Ikebukuro, Kiyohara and Sode, 2004)^[23] with an electrochemical variant was developed to detect parasites, utilizing "signal-on" architecture. "Initiated for detecting various parasitic organisms, electrochemical aptasensors successfully recognized only a few species."

4.1 Malaria by *Plasmodium falciparum*

Accurate malaria diagnosis is in high demand. Detection methods for pan-malaria and *Plasmodium falciparum* involve catalysis by specific enzymes: *Plasmodium* lactate dehydrogenase (PLDH) for pan-malaria and *Plasmodium falciparum* glutamate dehydrogenase (PFGDH) for *Plasmodium falciparum* (Singh *et al.*, 2019). Ogunmolayusi *et al.*, 2022^[42] reported the use of the rapid and cost-efficient paper-based analytical device (μ PAD) to detect the malarial biomarker: recombinant *Plasmodium falciparum* lactate dehydrogenase rPFLDH using surface immobilized aptamers. *Plasmodium* lactate dehydrogenase (PLDH) is a valuable malaria biomarker due to its high expression in *Plasmodium* cells, significant structural and kinetic differences from human enzymes, and unique amino acid sequences across *Plasmodium* species, enabling precise species identification within different samples (Jain *et al.*, 2014)^[24]. Frith *et al.*, 2018^[14] reported that rLDH7 aptamer has a 40nM dissociation constant (Kd) for the binding interaction with rPFLDH using microplate ELONAs. APTEC sensors combined with rLDH7 aptamer gave a higher detection limit of 16.6 nM than Malstat-based PLDH

capture assays (Ogunmolayusi *et al.*, 2022)^[42]. Conventional Rapid Detection Tests (RDTs) of *P. falciparum* were done based on PLDH and histidine-rich protein II (PfHRP2) (Ugah *et al.*, 2017; Dalrymple *et al.*, 2018)^[60, 10]. Lo *et al.*, 2021 reported 2106s SSDNA aptamer as the perfect candidate that can be combined with an electrochemical aptamer binding (E-AB) sensor to bind with higher sensitivity and specificity with PfHRP2. Both pLDH and HRP-II are significant biomarkers for detecting malaria infection. While pLDH is present in all malaria species, HRP-II is specific to *P. falciparum* parasites (Jain *et al.*, 2014; Howard *et al.*, 1986)^[24, 21].

4.2 Leishmaniasis

Leishmaniasis, a major tropical disease caused by *Leishmania* protozoa, is prevalent in Asia, Africa, and Central America. Female sandflies of the *Phlebotomus* and *Lutzomyia* genera transmit the disease by injecting promastigotes into the bloodstream. Sandfly saliva, containing anti-clotting and vasodilatory factors, aids infection. This disease affects humans and about 70 other animal species. Approximately 350 million people are at risk, with 0.7 to 1.2 million new cases reported annually (Burza, Croft and boelaert, 2018; Steverding., 2017). Bruno *et al.*, 2014 emphasized the diagnostic potential of the kinesin protein from *Leishmania* flagella for detecting Leishmaniasis. Using the RNA aptamer K2-13R, they successfully identified a kinesin protein band of approximately 250 kDa. Moreno *et al.*, 2011^[37] employed an aptasensor approach to detect the *Leishmania Infantum* kinetoplastid membrane protein 11 (LiKMP-11). These findings underscore the diverse applications of aptamers in advancing diagnostic methods for Leishmaniasis.

5. Diagnosis of cancer

Cancer is a significant public health issue and the second leading cause of death globally. The incidence of new cancer cases is expected to rise quickly due to population growth and ongoing lifestyle choices that elevate cancer risk (Siegel, Miller, and Jemal, 2018)^[50]. Hussain and Nguyen, 2014 emphasize that prioritizing early cancer detection and precise staging is just as vital as developing new anticancer medications and treatments for curing the disease. Aptamers have recently become promising molecular probes for enhancing the specificity and accuracy of cancer diagnostics and therapeutics due to their unique properties. Therefore, integrating aptamer-based technologies with physical or chemical diagnostic agents could significantly advance cancer detection (Kim *et al.*, 2018)^[27]. Cell membrane proteins like HER2 are often over expressed in various

cancers, leading to poor prognosis, suboptimal treatment outcomes, and lower survival rates. Numerous HER2-targeted drugs have been developed to treat tumors with high HER2 expression, effectively reducing HER2 levels. HER2-targeting DNA aptamers, known as Heraptamers, were labeled with ¹⁸F and evaluated both in vitro and in vivo for positron emission tomography (PET) imaging of HER2 in ovarian cancer (Zhu *et al.*, 2017) [70]. Chi-hong *et al.*, 2003 [66]; Thiel *et al.*, 2012 [57]; also reported Heraptamers for detecting HER2 expression in several cancers. Several aptamers, including SYL3C (Song *et al.*, 2013) [54], TuTu22 (Wang *et al.*, 2014) [62], EpCAM (Xiang *et al.*, 2015), Td05 (Ho *et al.*, 2015), sgc8-c (Sicco *et al.*, 2020) [66], and AS1411 (Fu & Xiang, 2021) [67], have been identified to bind to various molecular targets on cancer cells. Aptamer-based techniques for analyzing cancer cells are recognizing the molecular targets and capturing cancer cells, imaging and regulating membrane proteins with aptamers, and using aptamer-based probes for bioimaging at the subcellular level. Zeng, Tung and Zu, 2014 [69] developed a cancer cell-activatable probe by linking aptamers with paired fluorochrome-quencher molecules for a one-step assay of circulating tumor cells (CTCs), offering a valuable index for assessing tumor progression. Du, Peng and Li, 2019 [12] developed a lysosome-targeting framework nucleic acid (FNA) nanodevice that incorporates an i-motif and an adenosine triphosphate (ATP)-binding aptamer (ABA) into a DNA triangular prism. This nanodevice undergoes a conformational switch upon entering the lysosomal compartment of cancer cells, producing fluorescence to indicate abnormal pH and ATP levels, enabling dynamic imaging of the lysosomal environment.

Conclusion

Aptamer-based diagnostic methods have become increasingly promising in contemporary laboratories. Research across biomedical fields like bacteria and viruses, requires the help of aptamers in evaluating drug effectiveness, disease diagnosis, and therapy. SELEX-derived technologies facilitate innovative detection, diagnostic, and treatment methods, which are increasingly recognized for their advantages as alternatives or complementary to antibody reagents (Chen *et al.*, 2022) [6]. Aptamers have shown significant potential by enhancing existing detection methods and serving as novel biosensors for imaging and detecting cancer cells (Zhou *et al.*, 2017; Li *et al.*, 2014) [71]. Furthermore, there is a crucial need to expand the detection capabilities of aptamer visualization. Accelerating the application of nucleic acid aptamers in disease detection, precise diagnosis, and treatment is essential (Ospina-Villa *et al.*, 2016) [43]. It is crucial to advance ongoing research by optimizing the existing aptamers and prioritizing the development of new aptamers targeting novel molecular targets. Therefore, the aptamer-based technologies discussed in this review contribute comprehensive insights into viral, bacterial, protozoan, and cancer disease diagnosis.

Author Contributions

Writing—original draft preparation, S.B., S. L; writing—review and editing, S. L, S.B.; Images-supervision- S.B. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

Funding information

No funding has been received.

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