

# APTAMER TECHNOLOGY TO COMBAT ANIMAL DISEASES AND ITS FUTURE APPLICATIONS

## Abstract

Aptamers are synthetic single stranded DNA'S or RNA's that bind to target molecule with high affinity. Aptamers are developed using a special process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). The target of aptamers includes proteins, cells, microorganisms, chemical compounds, heavy metal ions, etc. Technological advancements in the fields of high-throughput sequencing (HTS), bioinformatics, microfluidics, and nanodevices have expanded the application of aptamers to make important contributions to both human as well as veterinary medicine. Earlier the disease was often diagnosed through separation from culture and propagation, both of which took time and effort to complete. present-day molecular diagnostic technologies, such as RT-PCR, PCR offer great specificity, sensitivity and speed for detection of pathogen but they also call for isolated genetic material, cautious handling and expensive equipments. But nowadays, aptamers can be used in the diagnosis of many bacterial, viral and protozoal diseases of veterinary importance in field conditions in a short period of time.

**Keywords:** SELEX, aptamer, aptasensors, aptamer conjugates, diseases, therapeutics.

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## I. INTRODUCTION

Aptamers are oligonucleotide sequences of between 25 and 80 bases in length that have the capacity to attach to particular target molecules. They act as a rival to the monoclonal antibodies and hence, are sometimes called as ‘antibody mimics’. They are small molecules with a size of about 6-30kda. They fold into various three-dimensional structures that bind to certain targets. The word "aptamer" is a combination of the Greek words ‘meros’, which means section or region, and the Latin word ‘aptus’ which means fit. Aptamers are isolated using a process called Systematic Evolution of Ligands by Exponential Enrichment. This method was first developed 30 years ago in 1990 by two independent labs-the lab of Larry gold who used SELEX for selection RNA ligands against T4 DNA polymerase. The other lab was of Jack Szostak who used SELEX for selecting RNA ligands against various organic dyes. Aptamers are becoming a good rival to monoclonal antibodies due to their several advantages over antibodies including ease of creation, low cost of production, little batch-to-batch variations, reversible folding capabilities, and very low immunogenicity.

**Table 1: Comparison between aptamer and monoclonal antibodies**

<b>Properties</b>	<b>Aptamer</b>	<b>Monoclonal Antibody</b>
<b>Structure</b>	Chain of oligonucleotides	Chain of amino acids
<b>Stability</b>	More stable as can withstand repeated rounds of denaturation/renaturation. Stable at room temperature.	They get easily denatured.
<b>Size</b>	Small molecules(6-30kda)	Large molecules(150kda)
<b>Tissue uptake</b>	Fast	Slow
<b>Kidney filtration</b>	Rapid due to small size	Slow due to large size
<b>Preparation</b>	It is in-vitro, takes only 2-8 weeks	It is in-vivo and takes long time of around 6 months or more
<b>Shelf life</b>	Long shelf life(several years)	Short shelf life
<b>Cost of production</b>	Cheap to synthesize	Expensive to synthesize

## II. SELEX PROCESS

SELEX is a gold standard process for the production of nucleic acid aptamers. It is a selection cycle for DNA or RNA sequences that mainly involves four steps- (i) selection of the library of nucleic acids of randomized sequence. (ii) incubation of a target molecule with this library. (iii) partitioning and retrieval of bound sequences from non-bound sequences. (iv) Recovery and amplification of the sequences. Amplification is done using PCR for DNA aptamers and RT-PCR for RNA aptamers. And this selection cycle is repeated until more than 90% of the sequences bind to their target molecule, this is known as enrichment. Thus, an enriched and dominating population of aptamer library species is created by iterative selection cycles that include binding, partitioning, recovery, and amplification stages.

### III. CHEMICAL MODIFICATION OF APTAMERS

Although aptamers have many benefits but their use in in-vivo is restricted due to some renal filtration. To overcome these issues some chemical modification is done on aptamers to provide more stability to their structure. These modifications involve:

- Increasing their molecular mass (6-30kda) by attaching some bulky moieties like polyethylene glycol (PEG), cholesterol at their 5' end to overcome rapid renal filtration. This will increase their circulation time.
- Modification of the sugar ring of the nucleoside.
- Modification at the ends of nucleic acid chain.
- Modification of the phosphodiester linkages to stabilize chains of nucleic acids by replacing conventional phosphate (PO) backbones with sulphur containing phosphate ester bonds.

### IV. APTAMER AGAINST VIRAL PATHOGENS

- 1. Foot and Mouth Diseases (FMD):** FMD is highly contagious vesicular disease of domestic and wild cloven footed animals. The causative agent is a small, non-enveloped, positive-sense, single stranded RNA virus belonging to the genus Aphthovirus of the family Picornaviridae called foot-and-mouth disease virus (FMDV). Several immunoassays like RT-PCR have been used as a diagnostic tool for FMDV but there is no portable field test for virus serotypes. An attempt was made to develop a competitive fluorescence resonance energy transfer (FRET)-aptamer based strategy for detection of foot-and-mouth (FMD) disease that can give results in minutes [1]. In this new approach a 14 amino acid peptide of VP1 protein of FMDV which is responsible for capsid formation was labelled with Black Hole Quencher-2 (BHQ-2) dye and was allowed to bind to FMD DNA aptamers which were labelled with Alexa Fluor 546-14-dUTP. The study found that at levels of 25–250 ng/mL of FMD peptide, a discernible, highly sensitive reaction could be seen within 10 minutes. Another approach was made to inhibit the viral replication. 3D pol is responsible for viral genome replication. In another study 3 aptamers (F38, F47 and F52) were used that selectively binded to 3D pol and inhibit the replication of FMD virus genome [2].
- 2. Avian Influenza:** Avian influenza also called bird flu is a serious threat to animal as well as human health. Influenza virus belongs to the family Orthomyxoviridae. Hemagglutinin (HA), a glycoprotein expressed on the viral surface, is a suitable target for aptamer-based antiviral therapy. An attempt was made to develop an aptamer (HAS15-5) based antiviral therapy against H5N1 virus which acts by blocking and inhibiting the receptor binding domain of viral hemagglutinin. In order to achieve the highly selective and sensitive amperometric detection of H5N1 virus proteins using a gold nanoparticle (NP) modified electrode, a highly sensitive sandwich assay platform was designed using a surface generated aptamer-protein-antibody complex [3]. This micro fluidic-based aptasensor can identify the H5N1 avian influenza virus as tiny as 0.0128 hemagglutinin units within 30 minutes in a field setting. This sandwich based aptamers were also used for the diagnosis of H5N2 whole viral particles. Lateral flow strips of graphene-oxide-based SELEX (GO-SELEX) were used which could detect the particles as low as  $6 \times 10^5$  in the buffer.

3. **Bovine Viral Diarrhoea (BVD):** BVD is significant infectious diseases of livestock worldwide caused by pestivirus of Flaviviridae family. Once the virus has invaded a herd, it spreads quickly, and the entire herd is sick within a few weeks. Therefore, it is essential to quickly detect and identify the BVD virus to prevent BVD epidemics in the herd. In order to create ssDNA aptamers that can selectively bind to a full BVDV type 1, an improved graphene oxide (GO)-based immobilization-free SELEX technique was used [4]. This approach uses two distinct aptamers with great specificity for BVDV Type 1. The first aptamer is a capturing aptamer that binds with the whole BVDV-1. The second aptamer is called the reporting aptamer and is conjugated with gold nanoparticle. This also binds with the BVDV-1 and forms a sandwich type -capturing aptamer-Whole BVDV Type 1- AuNP labelled reporting aptamer. This method can sense as little as 800 copies/ ml of virus [4].
4. **Infectious Bovine Rhinotracheitis (IBR):** IBR is a viral disease that affects both young and old cattle and is extremely infectious and contagious. Clinical symptoms of the illness include dyspnea, pyrexia and conjunctivitis. The causative agent is Bovine herpes virus-1, which also causes infectious pustular vulvovaginitis in the female and infectious balanoposthitis in the male and can lead to abortions and foetal deformities. A study was carried out to develop aptamers that can inhibit the BoHV-1 entry into the cells [5]. After eight iterative rounds of SELEX against purified immobilized BoHV-1, nine aptamers were selected. Out of those IBRV-A4 exhibited the greatest binding affinity and specificity for BoHV-1.

## V. APTAMER AGAINST BACTERIAL PATHOGEN

1. **Salmonella:** Salmonella is a one health pathogen. Salmonellosis is clinically manifested as a syndrome of septicemia, typhocolitis, acute or chronic enteritis and abortion in cattle, sheep, pig and horse. Different serotypes of Salmonella affect different species of animals for e.g. Salmonella enterica subspecies enterica serotype Dublin (*S. dublin*) and Salmonella enterica subspecies enterica serotype Typhimurium (*S. typhimurium*) most commonly affect bovine. In poultry also *S. typhimurium* causes fowl paratyphoid. *S. typhimurium* is a major cause of human salmonellosis that leads to a wide range of human health problems such as diarrhoea, bacterial fever (typhoid), food poisoning, stomach flu and infectious diseases of the urinary tract, lung and kidney. Several *S. typhimurium* isolates have evolved resistance to standard antimicrobials, including ciprofloxacin and cephalosporins, in industrialized countries, in addition to the public health problems associated with *S. Typhimurium* [6] and it is a matter of grave concern. A mixture of outer membrane proteins (OMP) was used as the positive selection target to select DNA aptamers against *S. enterica* serovar Typhimurium [7]. After being mounted on magnetic beads, these aptamers were used to collect the pathogen from biological samples for later PCR detection. The outer membrane protein, OmpC, of *S. typhimurium* was the target of several modified 2-F-RNA aptamers [8]. Recently, a QCM based aptasensor was also developed to detect *S. typhimurium*. This aptasensor could detect 103CFU/mL of *S. typhimurium* with less than 1h [9].
2. **Mastitis:** Mastitis is caused by a number of microorganisms like *Mycoplasma bovis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Mycoplasma bovis* mastitis is a highly contagious condition and leads to severe drop in milk in production. P48 protein

acts as an ideal biomarker for *M. bovis* and can be used as a diagnostic tool to detect the diseases. A competitive enzyme linked aptamer assay for the detection of *M. bovis* in sera was developed in which a single stranded DNA aptamer with excellent affinity and specificity against the P48 protein of *M. bovis* was utilized [10]. Another study used ELONA (Enzyme-Linked OligoNucleotide Assay) for the detection of Protein-A binding aptamer in *Staphylococcus aureus* [11]. Additionally, utilizing a glassy carbon electrode, an impedimetric aptasensor for the ultrasensitive detection of *Pseudomonas aeruginosa* was created. Silver nanoparticles were electro-deposited on the glassy carbon electrode to modify it, and NH<sub>2</sub>aptamer was covalently bonded to the electrode's surface. This could detect a range of 10<sup>2</sup>–10<sup>7</sup> CFU/mL of *P. aeruginosa*.

## VI. APTAMER: FUTURE APPLICATION IN ANIMAL DISEASE DIAGNOSIS AND THERAPEUTICS

**1. Aptamer Based Biosensors:** Aptasensors are a class of biological sensors and they incorporate aptamer as a recognition element which is conjugated to a transducer which converts the biological interaction between aptamer and target into legible signals. Aptasensors help in early diagnosis of different types of cancers and tumors. Quantum dot based aptamers for florescent imaging were used for circulating tumor cells. The aptamers were labelled with BHQ-2 and Cy5.5 fluorescent dye and were conjugated to quantum dots. To find analytes in complicated matrices like serum, living cells, food, or environmental materials, graphene aptasensors have been studied. Graphene is an ideal sensing platform for in situ analyte monitoring because it can prevent aptamers from being degraded by nucleases. The colorimetric aptasensor mechanism can be used for selective determination of drug abuse which is based on a unique interaction between target analyte and aptamer. Aptamers have also been used for the purpose of detecting drug remains in milk, meat and farm waste. A attempt was made to develop a DNA aptamer-based electrochemical aptasensor to detect sulfadimethoxine in milk [12]. A DNA aptamer that is unique to sulfadimethoxine was created, and when it binds to sulfadimethoxine, it causes nuclease PI to begin cleaving DNA, which results in an electrochemical change that can be detected by an electrode and could detect the sulfadimethoxine with range of 0.1–500 nmol/L.

Aptamers can also be used for sensing toxins present in feed and fodder which when consumed by animals can cause toxicity and death. One of the examples is ricin toxin present in castor bean. Ricin is highly thermostable and is also stable at acidic and alkaline pHs. With a detection limit of 25 ng/mL, aptamers against the ricin B-chain were demonstrated to be more effective than ELISA kits at detecting ricin B in a variety of liquid foods.

**2. Aptamer Conjugates:** Aptamer-conjugates are the future of therapeutics against animal diseases. Many antibiotics are hydrophilic, which limits their ability to enter cells. Antibiotics that accumulate in lysosomes lose bioactivity and limited intracellular activity against delicate bacteria is the outcome of this. Aptamers can be conjugated with some nanoparticles like liposomes which are preloaded with therapeutic agent against the specific pathogen. This nanoparticle based drug delivery system can release high concentrations of the therapeutic agent at the site of infection, while keeping the administered drug dose low. This conjugation helps in increasing the circulation time of

the drug. Liposomal formulations have been employed in numerous bacteria, including *Staphylococcus aureus*, *Salmonella* species, *Brucella* species, and *Mycobacterium* species, to treat animal ailments [13]. It was shown by MacLeod and Prescott that gentamycin liposomally entrapped can kill *Staphylococcus aureus* causing mastitis in cattle. Aptamers can also be conjugated with small interfering RNA (siRNA), small hairpin RNA (shRNA) and microRNA (miRNA) for targeted delivery of the therapeutics for RNAi-based gene therapy. This was mainly used for human diseases earlier but now a short-hairpin RNA (RNAi-VP4) targeting viral VP4 gene has been observed to prevent FMDV infection in primary epithelium cells of transgenic bovine fetus [14]. Several siRNAs against VP1 protein have been observed to act as potent inhibitors of FMDV replication in different cell lines.

## VII. CONCLUSION

The SELEX -aptamer technology can be used quickly and affordably. It can be used for on-site detection of pathogen in a cost effective way. Due to their careful integration with nanotechnology, microfluidics, and next-generation sequencing technologies, aptamers are emerging as viable tools for pathogen detection, illness surveillance, and disease therapy. It can be applied to the creation of efficient treatments for animal ailments. The time has come to create portable, miniature, sensitive, and focused diagnostic equipment that field veterinarians, epidemiologists, or competent authorities can utilize with ease to manage animal disease as conventional diagnostic methods require time, trained professional and sophisticated instruments. Therefore, the discovery of aptamers opened up new possibilities for clinical diagnostics as well as biosensing.

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