

Advances in Aptamer-Based Nanodiagnostics for Microbial Sensing

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Abstract

Accurate and rapid diagnostics are required for environmental monitoring, food safety, and medicine in the 21st century. Disposable sensors provide low-cost, user-friendly options for rapid measurement from clinical diagnostics to food science. Synthetic single-stranded DNA or RNA, termed aptamers, are relatively new tools with clear advantages in terms of structural, binding specificity, chemical flexibility, and new improvements in robustness in complex, regulated matrices. This review provides a comprehensive overview of nanomaterial-integrated aptamer-based biosensors, categorizing major diagnostic methods such as colorimetric, electrochemical, fluorescence, lateral flow, and CRISPR-based detection systems. Applications in clinical diagnostics, food safety, and environmental monitoring are addressed. Target accessibility, stability *in vivo*, and regulatory limitations are highlighted as the major challenges through the integration of recent studies. The current work aims to help researchers with designing and using long-lasting, aptamer-based nano biosensors for practical diagnostic applications.

Keywords: Aptamer, Nanomaterial, Biosensor, Microbial Diagnostics

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INTRODUCTION

Single-stranded nucleotides of DNA or RNA that target a specific region in proteins, small molecules are known as aptamers, which have a strong affinity towards the target.¹ They are artificially produced by a process called SELEX (Systematic Evolution of Ligands by EXponential enrichment), a sequential enrichment method used to isolate highly specific aptamer candidates from a whole set of aptamer sequences.² In recent years, applications against complex targets, like microbial pathogens, have been diagnosed with multiple complex SELEX variants, notably cell-SELEX, microfluidic-SELEX, and toggle-SELEX, which drastically improved the efficacy and specificity of aptamer selection.^{3,4}

Aptamers are more viable alternatives to antibodies in diagnostics and therapeutics because they possess strong affinity, remarkable specificity, and inherent stability. Aptamers can be chemically modified to introduce novel functionalities, enhancing their adaptability to different diagnostic strategies for identifying molecular targets. DNA and RNA aptamers contain multiple conformations that can provide higher-affinity binding, while they contain the best separated and composed common nucleic acid motifs. Unique structures also arise, including G-quadruplexes, stacked structures formed by four guanine bases linked via Hoogsteen-type hydrogen bonding. The first observed G-quadruplex was from a DNA-based aptamer designed to bind and suppress thrombin activity, exhibiting a compact, symmetrical structure that consisted of two guanosine tetrads and three loops.⁵ Predicting the secondary structures of aptamers is difficult, and particularly so for DNA aptamers containing G-quadruplexes or pseudoknots. Despite their structural intricacies, aptamers demonstrate excellent shape complementarity with protein epitopes, unlike conventional antibodies.

The structural flexibility of the aptamers plays a vital role when binding to the target, which can be enhanced by pairing the aptamers with functional nanomaterials like gold nanoparticles, carbon nanostructures, and quantum dots, which enhance the detection and improve biosensing applications, which is extensively studied for microbial diagnostics by enabling rapid, accurate

and affordable detection methods in contrast with conventional antibody-based techniques.^{6,7}

Aptamers utilize both well-defined nucleic acid motifs and structurally flexible regions to achieve high specificity and affinity for their targets. Upon interaction with their targets, aptamers can undergo conformational transitions while retaining specificity.^{8,9} Macugen stands as the first aptamer-derived therapeutic to receive FDA approval based on an aptamer to treat age-related macular degeneration.^{10,11} Recent efforts are focused on understanding aptamer structural features and how the properties of the aptamers can be used. Computational approaches for modeling aptamer 3D structures and simulating target interactions have proven valuable tertiary structures of aptamers and in docking them with targets, have also helped toward rational design.¹²⁻¹⁴

SELEX

The SELEX process (Systematic Evolution of Ligands by Exponential Enrichment) forms the foundation of designing high-affinity nucleic acid aptamers to discriminate between a diverse array of target entities (Figure 1). The SELEX process was independently created by Tuerk and Gold initially and later by Ellington and Szostak in 1990. SELEX is a process of universal *in vitro* evolutionary technique used to select aptamers with increased binding affinity and specificity.^{15,16} The process is initiated with a highly randomized large-scale oligonucleotide library of 20 to 60 nucleotides with constant primer binding sites for subsequent amplification.¹⁷ The library will be incubated with the target molecules, ranging from small molecules to whole cells or complex biological structures.¹⁸ Unbound molecules are washed away, leaving affinity-binding aptamers bound with the target. Bounded sequences are eluted out and amplified by PCR, and thus progressively enrich the subsequent iterative rounds for high-affinity candidates.¹⁹

Recent technologies like microfluidics and high-throughput sequencing have been integrated to increase selection and efficiency at less time and labor costs conventionally incurred in SELEX.²⁰ It has to be noted, however, that native aptamers are prone to degradation *in vivo*, and therefore, the addition of chemical modifications

is needed to make them stable and increase their pharmacodynamics.²¹ The inherent flexibility of the SELEX process leaves space for the creation of new targets and secondary structures of aptamers, as seen in the evolution of aptamers that can bind to ATP but also to GTP.²²

Types of aptamers

Aptamers are developed from DNA, RNA, or synthetic XNA molecules and have various applications in research, biosensing, biotechnology, and medicine. DNA aptamers are the most well-studied and used aptamers due to their chemical structure, stability, and low cost of production. These DNA aptamers can fold into a three-dimensional shape when they bind with and identify the target; their folding kinetics is considered to be fast, which occurs in milliseconds, and they also possess similar properties to small proteins, while they possess a more structured transition state with few intermediate formations.²³ RNA aptamers are more tunable and flexible than DNA aptamers, which are more binding-capable, and they can bind to targets. Due to their compact form, they exhibit extremely low immunogenicity and are easy to modify. They also have many benefits over antibodies in diagnostics and therapeutics.²⁴ RNA aptamers have also been promising in targeted delivery systems, such as the delivery of small interfering

RNAs (siRNAs) to target cells.²⁵ Xeno-nucleic acids (XNAs) represent the newest aptamer technology frontier. Chemically modified backbones or sugars contain synthetic analogs of the natural nucleic acids, the XNAs. These offer greater resistance to nucleases as well as enhanced biostability. XNA aptamers may be generated by dedicated selection protocols such as cross-chemistry SELEX (X-SELEX), using mutated polymerases to enable the inclusion of modified nucleotides. Among the various XNA alternatives, threose nucleic acid (TNA) aptamers dubbed “threomers” have drawn interest because of their antibody-like fold mimicry and more rapid protein-binding kinetics mediated by aromatic side chains.²⁶

Aptamer-based diagnostic platforms

Colorimetric assays

Aptamer-based colorimetric biosensors have proven themselves to be potent agents for rapid, sensitive determination of a wide variety of targets from disease biomarkers to pathogens and small molecules (Table 1). The assays preferentially function by visible colorimetric change or change in absorbance, abolishing the requirement for complex instrumentation and allowing them to be deployed at the point of care (POC)²⁷ constructed one of the earliest systems based on polydiacetylene vesicles for detecting *E. coli* O157:H7, while showed the application of

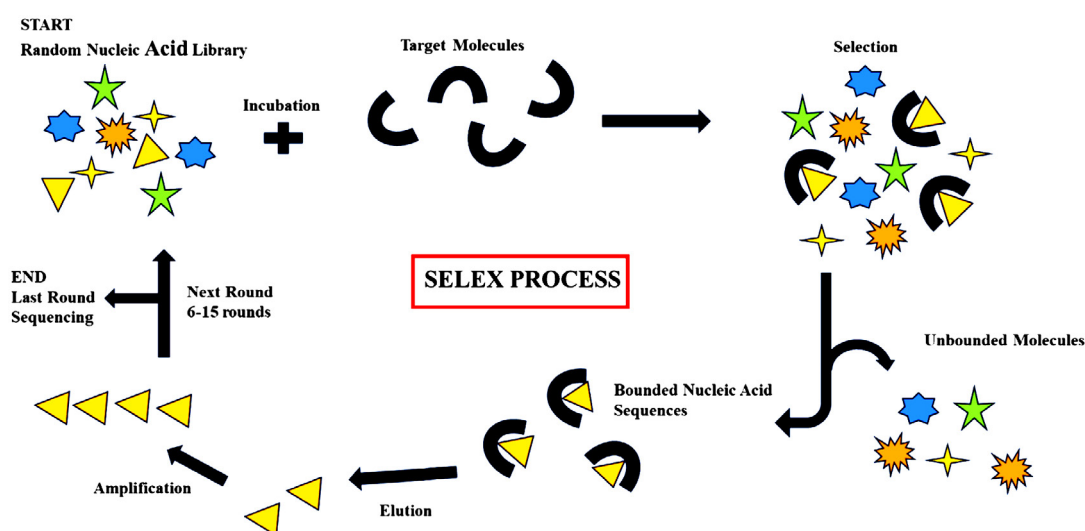


Figure 1. Overview of the Systematic Evolution of Ligands by Exponential Enrichment (SELEX)

gold nanoparticles (AuNPs) in the detection of thrombin and abrin toxin, respectively, as a sign of the versatility of aptamers with the help of nanomaterials.²⁸ Colorimetric assays are also used in food safety with high specificity and sensitivity in the analysis of foodborne pathogens reported higher than 90% sensitivity and specificity for several pathogens using low volumes of samples.²⁹

Aptamer target interactions with AuNP-based systems often result in nanoparticle aggregation or disaggregation due to variations in surface plasmon resonance, which causes significant color shifts. These assays frequently rely on their unique physicochemical qualities, and in some cases, these nanoparticles act as enzyme mimics (i.e., nanozymes), revealing intrinsic peroxidase-like activity. The presence of target molecules is determined by a catalytic process in which oxidation of chromogenic substrates takes place, which has the capability of showing visible color changes.^{30,31}

Fluorescence-based assays

Fluorescence assays are central to high-throughput screening platforms due to their sensitivity, automation facility, and wide range of fluorophores. Fluorescence assays have specific utility for the quantitation of enzymatic reactions, protein-protein and protein-DNA interactions, and cellular processes in real time. These technologies facilitate label integration directly into molecular targets with the maintenance of biological context and reduction of perturbation during measurement. In diagnostics and food safety, fluorescence technologies have sped up the identification of allergens, toxins, contaminants, and spoilage markers. Fluorescence microarrays and quantum dot biochips have been employed for the multiplex screening of trace residues and foodborne pathogens in parallel detection, ranging from picomolar to nanomolar.^{32,33} For instance, fluorophore-labeled probes have been utilized in a graphene oxide-based FRET platform to successfully detect aflatoxin B1 and *Salmonella* spp. in food samples with LODs below regulatory levels. Such technologies play a huge role in food traceability, authenticity, and public health guarantee.³⁴

FRET-based aptamer sensors

Fluorescence resonance energy transfer (FRET) is a universal and broadly used approach towards the construction of aptamer-based biosensors, where it utilizes the non-radiative transfer of energy from a donor to an acceptor fluorophore to generate highly sensitive and specific sensing signals. FRET has been successfully used in many applications, such as genetically encoded RNA-based FRET biosensors for the real-time monitoring of molecular interactions in living cells.³⁵ Further, upconverting phosphor-based FRET biosensors have shown superior sensitivity in the sensing of thrombin, a very significant coagulation disorder biomarker, by utilizing their unique photophysical properties to suppress background autofluorescence and boost signal-to-noise ratios.²⁷ In addition, the versatility of FRET-based aptamer sensors is also utilized in drug discovery research and live-cell imaging, through which they facilitate visualizing dynamic biomolecular processes and high-throughput screening of therapeutic candidates, thus driving both basic biological research and translational medicine.³⁶

Quantum Dot (QD)-aptamer conjugates

Quantum dot (QD)-aptamer conjugates are a novel family of biosensors that together take advantage of the unrivaled photostability and intense fluorescence of quantum dots and the high specificity and affinity of aptamer binding to provide them as powerful probes for cancer imaging and diagnosis. These conjugates have also been widely applied in both *in vivo* and *in vitro* detection of cancer cells, thereby facilitating specific identification and tracking of the tumor cells with improved sensitivity.³⁷ QD-aptamer conjugates have also been incorporated into electrochemical cytosensors that facilitate selective identification of tumor cells by electrochemical signaling pathways and thus a quantitative and sensitive method of diagnosing tumors.³⁸ In addition to single-target imaging, multiplexed imaging applications with various QD-aptamer conjugates simultaneously image several cancer biomarkers and enable cancer diagnosis and research to be more precise and complete.³⁹

Signal amplification strategies

To achieve enhanced sensitivity for aptamer-based fluorescent biosensors, several new signal amplification strategies have been documented. They include the use of fluorescent nucleotide analogues whose binding to the target upon activation generates a sensitive and direct fluorescent signal directly correlated with the molecular recognition event.⁴⁰ Another innovative method employs whispering-gallery mode lasing in microspheres, which enhances fluorescence signals through resonant light confinement to significantly raise detection limits.⁴¹ Cation exchange reactions within nanoclusters also exhibit effective amplification by controlling fluorescence properties based on target occurrence to offer a robust signal enhancement process.⁴² Besides, multisignal double-stranded DNA (dsDNA) probes have been developed to yield amplified diagnostic signals through multiple fluorescent emissions, thereby improving sensitivity and enabling more accurate biomarker quantification.⁴³ In sum, these fluorescence signal amplification methods provide powerful agents for improving the applications.

Multiplex detection strategies

Multiplex fluorescent aptasensors are effective analytical tools for the simultaneous determination of multiple analytes, thereby enhancing diagnostic assay throughput and efficiency (Figure 2). For instance, FRET-based systems coupled with graphene oxide quenching have been successfully employed in detecting a variety of antibiotics, enabling sensitive and selective multi-target analysis.⁴⁴ In addition, hybrid structures with quantum dots, aptamers, and (AuNPs) have also been developed to detect a range of ions and small molecules with higher specificity using the unique optical features and signaling transduction abilities of these nanomaterials.⁴⁵ Colorimetric aptasensors using AuNPs further enhance the multiplex detection capability by providing simple and visual readouts, which are particularly valuable for rapid testing in the field.⁴⁶ Other than fluorescence and colorimetry, novel multiplex detection formats also include entirely electrochemical sensors, SERS-based systems, and paper- or microfluidic chip-based systems. These newer technologies hold significant potential for high-sensitivity foodborne contaminant screening

by integrating miniaturization, portability, and multiplex capability to meet the requirements of analytical needs in the real world.³⁴

Electrochemical aptasensors

Electrochemical aptamer-based biosensors, dubbed electrochemical aptasensors, have been extensively used as extremely sensitive detection devices in clinical diagnostics, environmental inspection, and food safety assessment.⁴⁷ These sensors function through target-induced conformational, configurational, or conductance modifications of the aptamer on the electrode surface, and the inclusion of nanomaterials in electrodes has substantially enhanced sensor sensitivity, electron transfer efficiency, and overall performance. The electrochemical readouts are divided into label-free and label-based techniques. The label-free techniques include cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS), which track target binding in real-time without supplementary labels, whereas label-based techniques use enzymes, redox mediators, or nanoparticles to amplify the electrochemical signal upon target binding, thereby increasing sensitivity.⁴⁸ These methods facilitate real-time monitoring of compounds in intricate biological fluids, providing continuous, *in vivo* detection of medications, metabolites, and heavy metals at high temporal resolution.⁴⁹

Lateral Flow Assays (LFA)

Aptamer-based lateral flow assays (LFAs) have emerged as robust platforms for point-of-use, low-cost, and fast diagnostics by combining the molecular recognition capability of aptamers with the convenience of lateral flow technology. Their capacity for chemical modification and conjugation into gold nanoparticle (AuNP)-based formats might enable high-performance assays for mycotoxins, environmental micropollutants, and low-molecular-weight molecules.⁵⁰ Optimized concentration of aptamer, appropriate choice of membrane, and adequate blocking are critical parameters in the achievement of ideal specificity and sensitivity. Other innovative methods, like Linkage Inversion Assembled Nano-Aptasensors (LIANA), which improve the conjugation between

the nanoparticles and aptamer using structured linker assembly, and Crossover-SELEX, which is a variant of SELEX, aim to enhance the target specificity and affinity through iterative selection among various targets and conditions, have substantially improved aptamer-nanoparticle conjugation and target binding efficacy in lateral flow assay (LFA) systems.⁵¹ The devices enable fluid manipulation, metering, mixing, transport, and detection within compact platforms optimized for particular applications.⁵² Digital microfluidics (DMF) is particularly noted to offer multiplexing with the potential for portable point-of-care diagnostic devices.⁵³ They have low sample demands and provide rapid on-site detection and are therefore highly suitable for pathogen surveillance and clinical diagnostics.⁵⁴

Integration with nanomaterials

Aptamer-nanoparticle hybrid biosensors have garnered huge attention for their excellent sensitivity, target specificity, and rapid detection rates for a wide variety of analytes.⁵⁵ Aptamers have

high affinity that serve as effective bioreceptors if they are immobilized on nanomaterials such as gold nanoparticles (AuNPs), carbon nanomaterials (CNMs), magnetic nanoparticles (MNPs), and plasmonic nanomaterials.⁵⁶ Aptamer-nanoparticle conjugates are also being developed as multifunctional platforms for diagnostics and therapeutics owing to their distinctive blend of high specificity, tunability, and biocompatibility.⁵⁷ The benefits of these hybrid systems include diminished immunogenicity, heterogeneous surface functionalization, and target molecular recognition (Figure 3).⁵⁸ Optimization strategies for these conjugates include control of nanoparticle size, chemical bonds, and multivalent aptamer architecture for increasing their functional yield. Overall, their integration is a valuable progression toward the creation of targeted and individualized therapeutic platforms with low off-target toxicity.⁵⁹

Gold Nanoparticles (AuNPs)

Aptamer-functionalized gold nanoparticles (Apt-AuNPs) are used for their

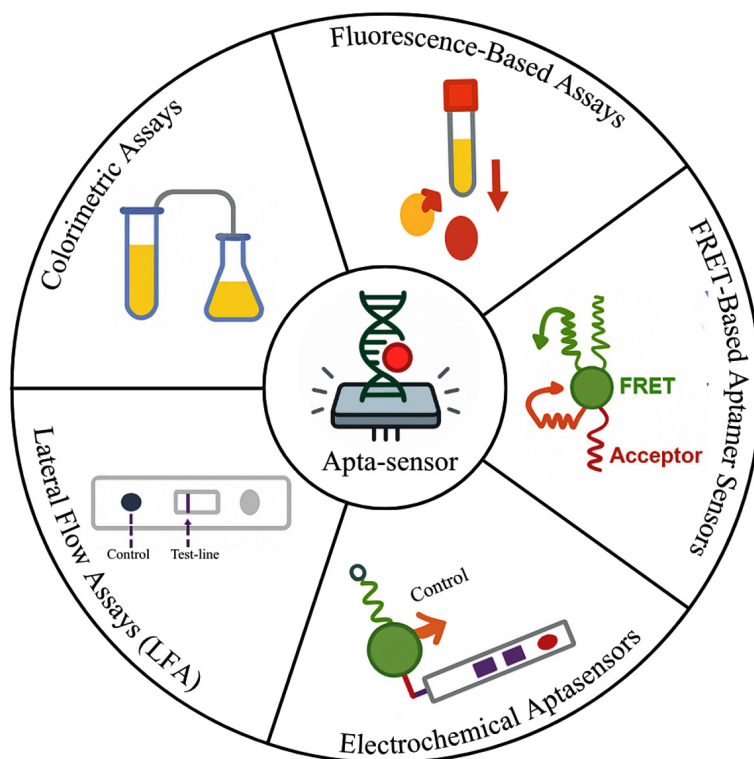


Figure 2. Aptamer-based diagnostics platforms

biocompatibility, ease of surface functionalization, and strong surface plasmon resonance properties.⁶⁰ These platforms have demonstrated exceptional utility in both colorimetric sensing and targeted drug delivery, offering rapid and sensitive detection capabilities with minimal instrumentation.⁶¹ For example, aptamer-induced aggregation of AuNPs has enabled simple colorimetric detection of organophosphate pesticides like malathion, with limits of detection as low as 1.48 µg/L within 40 minutes. Moreover, thiol-modified aptamers immobilized on AuNPs have shown strong stability and successful detection of glycated hemoglobin (HbA1c) directly in whole blood samples.⁶²

Carbon Nanomaterials (CNMs)

Graphene and carbon nanotubes (CNTs) significantly improve biosensor sensitivity by offering a high electroactive surface area, excellent conductivity, and ease of aptamer immobilization.⁶³ CNT-based aptasensors have reached femtomolar detection limits for targets like bisphenol A.^{57,64} Recent advances include reduced graphene oxide field-effect transistor (rGO-FET) sensors detecting NT-proBNP in artificial saliva at concentrations as low as 41 fg/mL.⁶⁵ Nonetheless, the complex three-dimensional structures of CNTs can present challenges in reproducibility and aptamer stability.⁶⁶

Magnetic Nanoparticles (MNPs)

Magnetic nanoparticle-based aptamer biosensors offer efficient separation and concentration of targets in complex biological

and environmental matrices.⁶⁷ These platforms have been used to detect as few as 10 cancer cells, with recent systems achieving detection of heat shock proteins like HSP70 with LODs as low as 0.1 ng/mL and multiple reuse cycles,⁶⁸ use in point-of-care diagnostics is expanding, supporting rapid, portable biomarker detection with high clinical relevance.⁵⁴

Plasmonic nanomaterials

Plasmonic nanomaterials, particularly AuNPs and AgNPs, enhance aptamer-based detection through surface plasmon resonance (SPR), self-catalytic growth, and metal-enhanced fluorescence.⁶⁹ Multispectral aptasensors utilizing plasmonic nanomaterials have demonstrated heightened bulk and surface sensitivity, enabling rapid detection of bacterial pathogens like *E. coli* O157:H7.⁷⁰ However, challenges in achieving multiplexing and clinical reproducibility remain significant.⁷¹

CRISPR Cas-based aptamer hybrids

The CRISPR-Cas systems have drawn attention to the development of more sophisticated diagnostic platforms. The plasticity and high sensitivity of aptamers and the programmable nuclease function of CRISPR-Cas effectors provide sensitive detection of small molecules with a wide variety of biomarkers^{72,73} constructed a molecular radar strategy using CRISPR-Cas12a for highly sensitive ATP detection²⁴ also constructed a CRISPR/Cas12a immunoassay with an aptamer-compatible platform, which is compatible with

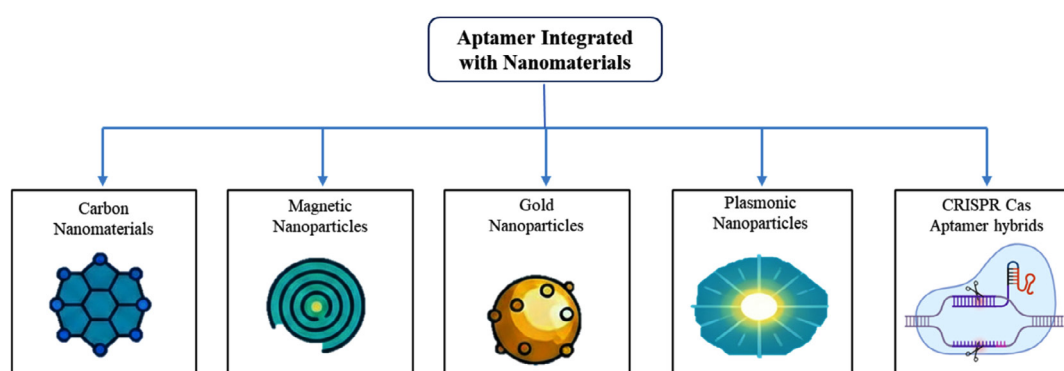


Figure 3. Aptamers integration with various nanomaterials

Table 1. Timeline of Breakthrough Innovations in Aptamer-Based Diagnostics

Year	Breakthrough	Detection type	Target Analyte	LOD	Ref.
2020	Aptamer functionalized porous silicon and carbon nanotube-based biosensors for protein detection	Electronic	Thrombin, his-tagged proteins	Single molecule, real-time detection	78
2021	Electrochemical aptamer sensor for neuropeptide Y with rapid dynamic measurement	Electrochemical	Neuropeptide Y (NPY)	162 pM (in buffer), 390 pM (in serum)	79
2022	Aptamer-based targeted drug delivery platforms in cancer therapy	Various (Therapeutic)	Cancer biomarkers	-	80
2023	Aptamer-functionalized molecular electronic device for continuous thrombin detection	Electronic/ Electrochemical	Thrombin	Single molecule detection	76
2024	Aptamer-based fluorescent sensor for rapid mercury ion detection in aqueous and living cells	Fluorescent	Mercury ions	33 µg/L (~0.16 µM)	81
2025	Peptide aptamer electrochemical sensors with antifouling layers for amino acid detection	Electrochemical	L-Arginine	0.01 pM	82
2025	Integration of functional nanomaterials (AuNPs, graphene, MOFs) to enhance aptamer sensors	Electrochemical	Prostate-specific antigen (PSA)	0.05 fM	4

conventional ELISA platforms, and achieved diagnostic versatility on non-nucleic acid targets. Applications also include clinical diagnostics, like Alzheimer's disease, for which designed a CRISPR-based aptasensor for the detection of Aβ40 and Aβ42 cerebrospinal fluid biomarkers with improved performance compared to standard ELISA.⁷⁴ Aptamer-CRISPR biosensors are noted for their simplicity, rapidity, and cost-effectiveness when compared to conventional assays.⁷⁵

Aptamer-based diagnostics application fields

Aptamer-based diagnostics have been acknowledged as powerful devices in a wide range of applications since they possess high specificity, high affinity, chemical stability, and easier synthesis than antibodies (Figure 4).⁷⁶ Despite problems of implementation with deeply rooted antibody use in commerce and poor public awareness, advances have opened up their usage across infectious disease detection, cancer diagnosis, environmental monitoring, and food testing safety. Diversiform platforms such as ELISA-like assays, lateral flow tests, microfluidics, and aptasensors have enhanced sensitivity and specificity for various diagnostic applications.⁷⁷

Infectious disease diagnostics

Aptamers are known to be far better than antibodies in bacterial and viral diagnosis; they have easier synthesis, modifiability, and wider target ranges.⁸³ Aptasensors, ELISA-type, and lateral flow devices are selective sensors that can detect pathogens like *Salmonella typhimurium* at 600 CFU/mL concentrations.^{84,85} Aptamers are utilized as bio-recognition units in aptasensors for point-of-care virus detection of HIV, HBV, HCV, SARS, influenza, and Ebola.^{86,87} Aptamers can interfere with virus-host interaction or intracellular protein binding to stop viral replication.⁸³ Aptamer biosensors combine optical, electrochemical, and mass-sensitive sensing modes for high-affinity and selective detection of bacterial proteins, surface antigens, and toxins.⁸⁸ Single-cell level detection is difficult, although combining nanomaterials and advanced SELEX methods holds the promise of increased sensitivity.²²

Cancer diagnostics

Aptamers, "chemical antibodies", have

been employed extensively in cancer biomarker detection and diagnosis of tumors because of their high selectivity, affinity, ease of synthesis, and chemical stability.⁸⁹ Cell-SELEX with proteomic analysis allows the simultaneous measurement of numerous cancer biomarkers.¹⁶ Aptamer-based biosensors (aptasensors) by electrochemical, optical, and fluorescence methods enable cancer early diagnosis to be performed rapidly, sensitively, and economically.⁹⁰ If combined with nanotechnology, there is further optimization of the sensor's performance. Aptasensors are also future candidates for point-of-care diagnosis and non-invasive tumor imaging, bypassing the limitations of traditional approaches.⁹⁰ Aptamers in therapeutics are used as drug delivery targeting ligands, where diagnostic and therapeutic functions are sheltered.⁹¹ Fluorescence, colorimetric, and multimodal imaging probes have been engineered in recent times for cancer detection and treatment approaches.²

Environmental monitoring

Aptamer biosensors are powerful, selective, and responsive environmental toxin sensors, for example, heavy metals, agrochemicals, and waterborne pathogens.^{92,93} SMART aptasensors are Specific, Measurable, Accurate,

Robust, and Time-saving sensors that enable rapid and sensitive analysis for food, water, and biological samples.⁷² Nanomaterial doping and heterogeneous transduction methods, optical, electrochemical, and mass-sensitive, mean specificity and sensitivity enhancement. Detection of pollutants such as pharmaceutical residues, endocrine disruptors, pesticides, and cyanotoxins is achievable by the biosensors with detection values upgraded to the femtomolar.⁹⁴ Wearable next-generation technologies and lab-on-a-chip platforms enable potential multifunctional environmental monitoring.⁹³ Problems remain in sensor sensitivity in sophisticated environmental matrices and online monitoring.⁹²

Food safety testing

Aptasensors also exhibit vast potential for the rapid, sensitive, and economical identification of contaminants and foodborne pathogens.⁶¹ Aptamers are employed in these biosensors to differentiate bacteria, toxins, antibiotics, and allergens in electrochemical, optical, fluorescence, and surface-enhanced Raman scattering detection modes.⁹⁵ Recent developments incorporate multiplexed arrays of detection and transcription aptasensors for label-free, culture-free whole pathogen detection.⁹⁶ Screen-printed electrodes-

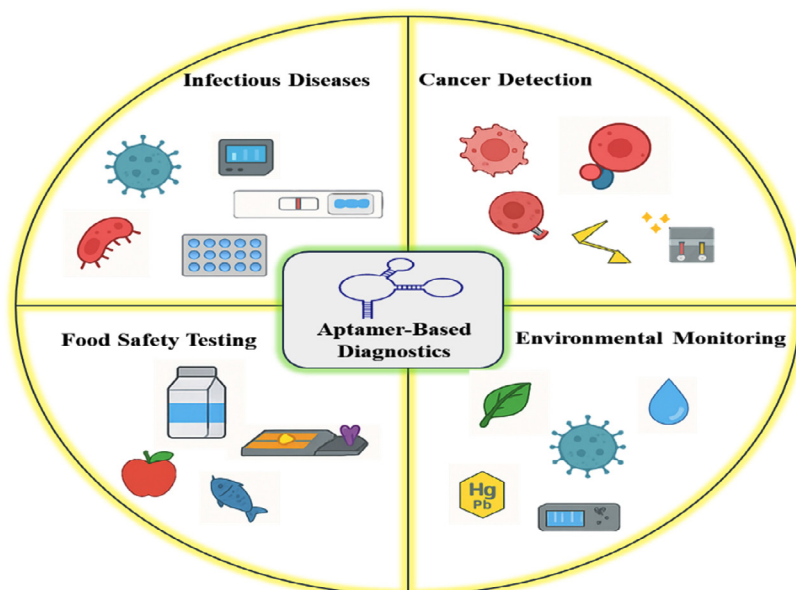


Figure 4. Applications of Aptamer-Based Diagnostic Platforms

Table 2. Summary of aspects, key challenges, and future prospects influencing the market readiness of aptamer-based diagnostics

Aspects	Key Points	Challenges	Future Scope
Market Readiness	Rapid growth in point-of-care and multiplex aptamer diagnostics (microarrays, QDs, CRISPR).	Limited clinical validation and standardization.	AI integration and portable platforms for faster, real-time deployment.
Regulation	WHO Essential Diagnostics List guides priorities; FDA/CLIA and EUA facilitate approvals.	Regulatory diversity delays global accessibility.	Development of harmonized, adaptive international approval systems.
Intellectual Property	IP encourages innovation; open models (CAMBIA, IMI) improve diagnostic accessibility.	Restrictive licensing limits equitable access, especially in LMICs.	Adoption of flexible or semi-commons IP models balancing innovation and access.
Accessibility & Cost	New diagnostics reduce costs and improve clinical outcomes.	High upfront costs and poor infrastructure in LMICs.	Promotion of local manufacturing and optimized supply chain models

based electrochemical aptasensors provide enhanced analytical performance in food matrices of multi-component.⁹⁵ Advantages of aptamer-based diagnostics in food safety are ease of modification, chemical stability, and *in vitro* synthesis over antibodies.⁹⁵ Future research remains to grapple with problems of strong detection in multiplexed samples and to offer useful applications along the food supply chain.⁹⁶

Constraints and challenges of aptamer-based diagnostics

Concerns of stability and shelf-life

Aptasensors, particularly electrochemical-based aptamer (E-AB) biosensors, have been refined with an enhanced understanding of their degradation processes. In contrast to earlier presumptions, signal decay is no longer largely due to nuclease hydrolysis, as late research indicates. Rather, sensor degradation now largely results from the loss of monolayer components on the electrode surface.⁹⁷ Thermostable analogs such as TNA aptamers are also superior to their conventional DNA and FANA counterparts concerning nuclease resistance and heat stability.⁹⁸ Storage stability has also been demonstrated that DNA aptamers are stable for more than two years under appropriate storage.⁹⁹ Electrochemical sensors preserved at -20 °C remain active for six months even without preservation.⁹⁴ Other methods, such as adapted SELEX and engineered polymerases, have increased the nucleic acid and peptide aptamer stability (Figure 5).⁹⁸ Structural characteristics such as loop size and center

sequence are also important for stability.⁹⁷ Taken together, these innovations supply essential information for constructing robust, stable diagnostic platforms.

Challenges with specificity and cross-reactivity

Aptamers offer a cost-efficient, targeted competitor to antibodies for biosensing applications.¹⁰⁰ But cross-reactivity is still at the root of diagnostic accuracy. Methods like aptamer blend optimization, where aptamers with different affinities are mixed, increase sensor selectivity without being confounded by interference.¹⁰¹ Cross-recognition methods, like those used with lateral flow tests for detecting small molecules, demonstrate aptamers' utility across a wide range of targets.¹⁰² Nevertheless, challenges with library design, post-SELEX modification, and screening sensitivity persist.⁹⁴ Technologies such as magnetic bead capture, flow cytometry quantification, and integration of transduction technology have resulted in ultrasensitive diagnostic devices that have reached commercialization.⁷⁵ Further development of these technologies is poised to make aptamers ready for widespread use for biomarker discovery and disease monitoring.

Production and standardization

While aptamers are replete with promise due to their specificity and modularity, practical problems of manufacture and standardization have to be addressed. Limitations such as modest *in vivo* efficacy, sparse safety data, and costliness of manufacture restrict their large-

scale application.¹⁰³ Furthermore, large-scale synthesis creates monstrous datasets, rendering clinical interpretation a problem.⁵⁰ Besides low batch-to-batch variation and ease of handling,¹⁰⁴ the SELEX success rate is still less than optimal. Improved approaches include microenvironmental tailoring and optimized post-SELEX procedures.¹⁰⁵ Overcoming these technological and scientific challenges is crucial to expanding the scope of applications for aptamer technologies in therapeutics, diagnostics, and drug delivery.

Diagnostic innovation: market, regulation, IP, and accessibility

Market readiness and technological feasibility

New diagnostic technologies propel forward to meet global health requirements, especially in resource-limited settings. Point-of-care (POC) diagnostics provide rapid clinical decision-making with minimal human interaction and are particularly valued for infectious and cardiovascular illnesses.¹⁰⁶ Multiplex analysis is enabled by high-throughput platforms, such as addressable laser bead immunoassays, microarrays, and nanobarcode particles, though clinical adoption requires validation and standardization.¹⁰⁷ Aptamer-based platforms have expanded diagnostic capability significantly, particularly when integrated with CRISPR/Cas platforms to enhance specificity and biosensing

ability.⁷² Aptamer-functionalized QDs have enabled multiplex imaging and drug targeting in cancer cells with improved tumor imaging and therapeutic performance.³⁹

Regulatory frameworks and diagnostic approvals

In vitro diagnostic (IVD) devices play a central role in disease detection and management. The Essential Diagnostics List (EDL) of the World Health Organization identifies the world's need for quality, affordable diagnostics.¹⁰⁸ Selecting quality diagnostics to use for low-resource settings involves process approaches, from defining use cases, market research, and monitoring performance.¹⁰⁹ In the United States, control is maintained by the FDA and CMS according to CLIA guidelines.¹¹⁰ Emergency Use Authorization (EUA) that was initially initiated in 2004 has been instrumental during health crises like COVID-19 by facilitating access to priority diagnostics and therapeutics.^{111,112}

Intellectual property and licensing considerations

Intellectual property (IP) policies are central to encouraging innovation and supporting access to the relevant diagnostics. Effective IP systems support biomarker research, technology commercialization, and international health partnerships.¹¹³ Open innovation strategies, including CAMBIA and the Innovative Medicines

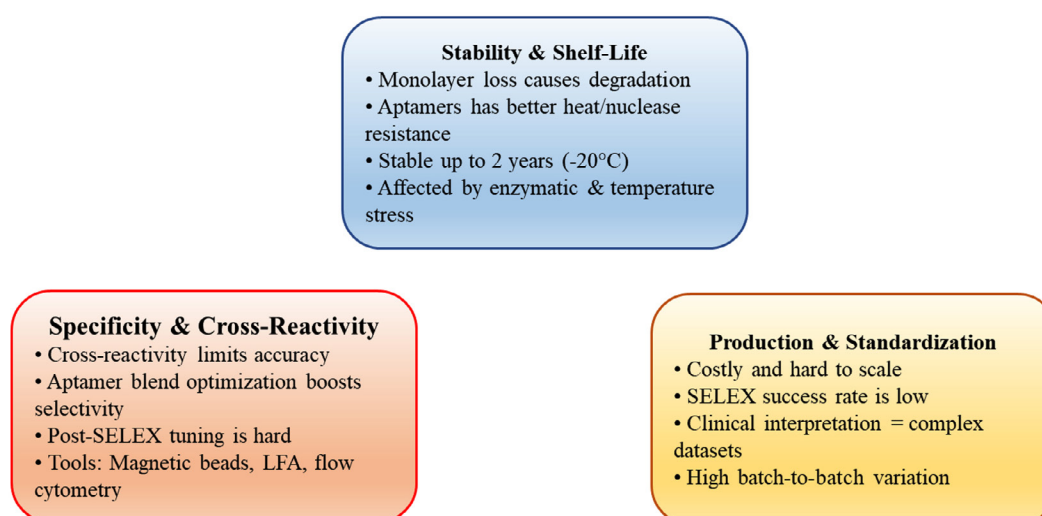


Figure 5. Key Challenges in Aptamer-Based Diagnostic Development

Initiative, are transforming corporate IP cultures through expanded accessibility.¹¹⁴ The CRISPR-Cas9 context has brought diagnostic IP licensing complexity into the limelight. Institutional ownership-commercial interest disputes have ignited controversy regarding ethical licensing models and semi-commons strategies that ensure equitable access and continued innovation.^{115,116}

Cost-effectiveness, scalability, and access

New diagnostics have shown promise for cost savings and clinical benefit in multiple situations. Some examples include results of triple testing for breast cancer and MRI usage for scaphoid fracture, both reducing unnecessary procedures and cost.¹¹⁷ Although NGS is more sensitive in oncology, this is coupled with higher upfront costs, balanced by more effective clinical trial pairing and results (Figure 6).¹¹⁸ As advanced as technology has become, access in LMICs is still inhibited by the environment, supply chain limitations, infrastructure, and affordability.¹¹⁹ Stock-outs at rural health clinics and poor supply chain coordination limit diagnostic delivery.¹²⁰ Enhancing access can be optimized by the likes of harmonized standards, dynamic supply chain models, resourced health systems, and quality assurance (Table 2).¹²¹

Artificial intelligence integration in aptamer-based diagnostics

Artificial intelligence (AI) significantly improves aptamer-based diagnostics by enhancing the development, efficiency, and statistical interpretation of aptamer systems that employ machine learning with deep learning algorithms, allowing the prediction and generation of aptamer sequences that have high affinity and specificity towards the target. Integrating sequence and structure-based parameters permits these computational models to precisely forecast aptamer-target interactions, thus minimizing experimental efforts and accelerating discovery and enhancing the analytical capabilities of aptamer-based biosensors, particularly when combined with nanoparticle-functionalized or electrochemical sensing platforms, thus improving signal processing, sensitivity, and accuracy.^{122,123} Advanced predictive modeling involving architectures such as transformers, variational autoencoders, and reinforcement learning algorithms has simplified in silico affinity optimization, assisting in the rational design of aptamers customized for personalized and point-of-care diagnostics.¹²⁴

AI-driven workflows enable rapid interpretation of high-throughput sequencing data by accelerating clinical decision-making

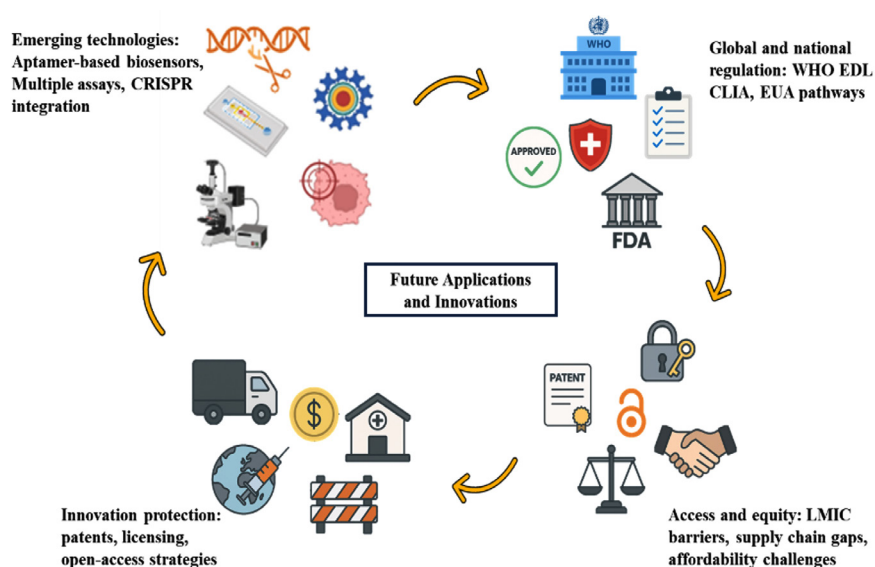


Figure 6. Strategic Roadmap for Future Aptamer Innovation

and improving diagnostic reliability with the integration of AI with modern diagnostics, including microfluidic device platforms, biosensors, and Internet of Medical Things (IoMT), which presents major opportunities for real-time, data-driven, and accessible healthcare solutions. Despite all developments, significant challenges in maintaining the stability of aptamers, large-scale production, and the need for regulatory standardization. Future research has to be carried forward on the development of standardized and transport AI procedures that assure us reproducibility, scalability, and fidelity for clinical requirements. This combination enables the growth of rapid, cost-effective diagnostic systems, which implies significant advancements in precision medicine.^{123,125}

CONCLUSION

Aptamer-based biosensors are becoming a universal and robust platform for contemporary diagnostics because of their tunable structure, easy chemical synthesis, and elevated specificity. Following conjugation with nanomaterials like gold nanoparticles, carbon nanostructures, quantum dots, and metal-organic frameworks, their functional performance is greatly improved in stability, signal transduction, and sensitivity. This review assessed significant detection approaches, colorimetric, electrochemical, fluorescence-based, lateral flow, and CRISPR-based systems, zealously illustrating how each modality is advantaged by the aptamer-nanomaterial synergy. Particular attention was placed on target recognition mechanisms, amplification approaches, and biosensor format innovation, making these systems available for point-of-care and on-site analysis. Technologies concerned have been extensively applied in the identification of pathogenic microorganisms, viruses, toxins, small molecules, and relevant biomarkers in medicine, the environment, and food safety. Although significant strides have been made, issues of aptamer stability in biological matrices, matrix interference, and mass manufacturing remain. Future work must focus on building stable *in vivo* aptamer systems, incorporating them into digital platforms and AI platforms, and multiplexed sensing. With the ongoing chain of interdisciplinary breakthroughs,

nanomaterial-aided aptamer sensors are bound to bridge the gap between molecular diagnostics in the laboratory and cost-effective, real-world health care and monitoring solutions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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