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Review Article

A comparative mini review on nanoparticle-based colorimetric sensors for detection of viral nucleic acids and proteins and future advancement using artificial intelligence (AI) and machine learning (ML)

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Abstract — Nanomaterial-based bio-sensing has established a new era of highly sensitive, specific, and accessible diagnostic technologies. The interface of engineered nanomaterials with advanced computational tools—particularly machine learning (ML) and artificial intelligence (AI)—has revolutionized biosensor design and functionality. Emerging platforms now integrate colorimetric sensor arrays, electrochemical interfaces, microfluidic systems, and CRISPR-based detection schemes to achieve ultralow detection limits, often in the attomolar (aM) to femtomolar range (fM), without the need for sample dilution. Molecular amplification strategies such as rolling circle amplification (RCA), loop-mediated isothermal amplification (LAMP), strand displacement reactions, and hybridization chain reactions (HCR) further enhance analytical performance. These technologies have robust performance in complex biological matrices like whole blood, serum, and saliva, thereby pushing diagnostics from the bench to real-world clinical and point-of-care settings. In this review, we discuss recent innovations in nanomaterial-enabled biosensors, emphasizing the integration of AI/ML for pattern recognition, signal optimization, and predictive analytics. We also highlight emerging diagnostic applications aimed at early disease detection, personalized medicine, and global health surveillance.

1. Introduction

A recent chapter in the book Nanomaterials Design for Sensing Applications explores how researchers design colorimetric sensors and sensor arrays. The chapter focuses on several important aspects, including receptor specificity, strategies to immobilize sensing molecules on solid surfaces, how sensors visually present results, and how analysts interpret these outputs [1]. Colorimetric sensors are analytical devices that detect target analytes through visible color changes, offering a simple and intuitive readout without the need for complex instrumentation. Unlike fluorescence-based assays that often require complex optical instrumentation and suffer from issues like photo-bleaching and background auto-fluorescence, colorimetric sensors generate visible color changes that can be detected with the naked eye or simple imaging devices, making them ideal for point-of-care and resource-limited settings. Compared to electrochemical sensors, which typically require

precise control of electrode interfaces and external power sources, colorimetric platforms are more straightforward and amenable to miniaturization without compromising user-friendliness.

Lately, scientists have increasingly turned to nanoparticles in biosensor design. These materials offer a high surface-area-to-volume ratio, minimal interference in biological environments, straightforward chemical modification, affordability, and rapid separation response. Gold and magnetic nanoparticles appear most frequently among the wide range of nanomaterials [2]. Nanoparticle based colorimetric sensors can detect extremely low concentrations of analytes and perform even better when researchers pair them with surface-sensitive transducers. This combination significantly boosts both selectivity and sensitivity [3].

Early nanoparticle-based biosensors primarily used the particle's intrinsic peroxidase-like activity or aggregation-based detection techniques [4]. Nanoparticles like gold, silver, iron oxide, cerium oxide, and various carbon-based structures can catalyze peroxidase reactions without needing traditional enzymes like horseradish peroxidase (HRP) [5]. These nanoparticles catalyze the decomposition of hydrogen peroxide (H_2O_2), producing reactive oxygen species. These species then oxidize specific substrates, generating colorimetric, fluorescent, or electrochemical signals that are easy to measure. Researchers have used this mechanism to detect bacteria [6, 7], DNA [8], single nucleotide polymorphism [9], and proteins [10].

However, aggregation-based sensors face significant challenges when analyzing complex biological samples. In these environments, nanoparticles often clump together uncontrollably, resulting in high background signals and the potential for false positives. To address these issues, scientists have developed gold nanorod-based plasmonic sensors that focus on changes in aspect ratio instead of aggregation. This innovative approach helps create clearer signals that are more reliable for detecting target analytes. These sensors create clear multicolor signals in response to various target analytes [1-13].

Magnetic nanoparticles have advantages over other nanoparticles. It gives a special ability to manipulate it with external magnetic fields, and researchers can selectively capture, isolate, and concentrate the molecules they are interested in. This feature enhances detection sensitivity and speeds up the testing process; therefore, magnetic nanoparticles play a crucial role in state-of-the-art biosensing. Magnetic nanoparticles can, likewise, be readily integrated into portable and automated biosensing platforms, which is of special interest for their application in the field [14, 15].

One advanced biosensor design combines rolling circle amplification (RCA) and DNA strand displacement (DSN) to detect microRNA. This sensor uses magnetic core-satellite nanostructures to boost both sensitivity and specificity. When users mix a sample containing microRNA with the appropriate reagents and incubate it, the system produces a signal indicating the presence of the target microRNA. This biosensor detects microRNA at concentrations as low as 1 femtomolar (fM), even in complex samples like plasma [16]. Another report used a similar RCA-based method with magnetic particle assembly to achieve detection in 10% serum environments for microRNA detection [17].

To push sensitivity even further, researchers combined different amplification techniques. One assay designed to detect microRNA-155 uses a dual-amplification strategy. It merges the hybridization chain reaction (HCR) with the peroxidase-like activity of copper nanoparticles (CuNPs). The process begins when an amine-functionalized probe (P1) binds to magnetic beads. When the target microRNA-155 binds, it links with a second probe (P2), creating a sandwich structure that initiates HCR. This results in a super-sandwich complex. CuNPs form within this complex and catalyze the oxidation of a chromogenic substrate (TMB), producing a measurable blue color. This method can detect microRNA-155 in human serum with an impressive detection limit of 22 attomolar (aM) [18].

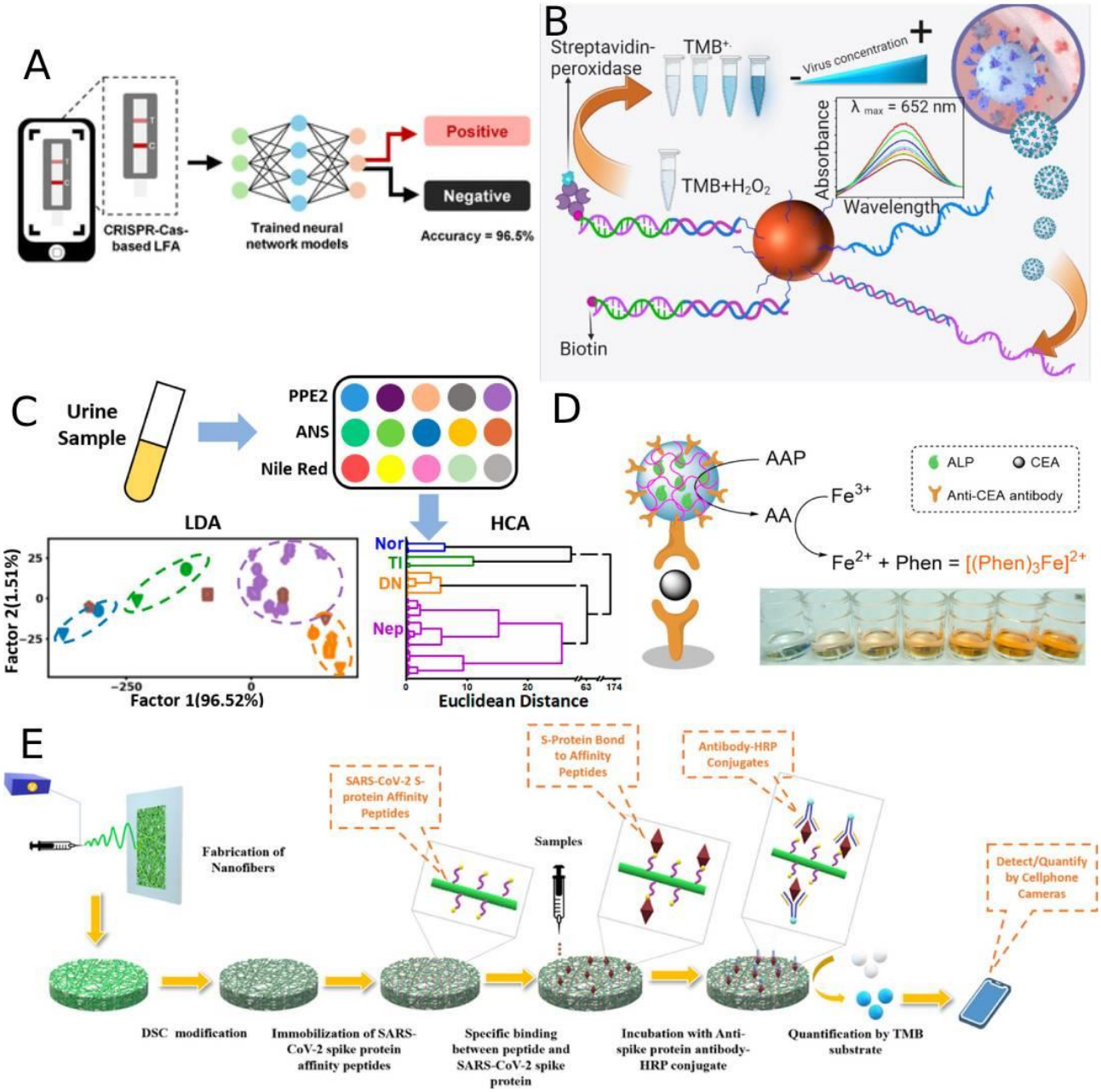

Figure 1. Colorimetric assay of various analytes with diverse techniques that are amplified with machine learning. **A)** Machine learning used to analyse CRISPR based Lateral flow assay **B)** Colorimetric Hybridization Sensor for DNA Mimic of a SARS-CoV-2 RNA Marker **C)** Fluorescence sensor array for discrimination of urine proteins and differentiation diagnosis of urinary system diseases **D)** A colorimetric immunoassay based on coordination polymer composite for the detection of analyte **E)** Highly sensitive detection of SARS-CoV-2 spike protein using a smart phone.

More recent studies continue to improve detection techniques by combining multiple approaches. One group paired magnetic nanoparticles with Cas13 and HRP labelling to detect the SARS-CoV-2 virus in serum samples, reaching femtomolar sensitivity. Another study used Cas12a to detect the SARS-CoV-2 N-gene without a colorimetric label, though this method only achieved nanomolar-level detection [19].

Table 1. Comparative analysis of nanoparticle-based sensors for the detection of various viruses.

Analyte	Label/type of particle	Method	LOD	Ref.
SARS-CoV-2	Gold nanoparticles	Aggregation	0.18 ng/μL	[4]
Campylobacter jejuni DNA genome	Gold/Platinum nanoclusters	Intrinsic Peroxidase-Like Activity	20 pM	[6]
Staphylococcus aureus	Potassium chloride-doped carbon dots/ magnetic multi-walled carbon nanotube composites	Intrinsic Peroxidase-Like Activity/ Fluorescence/ photo-thermal	4.81 cfu/mL, 3.40 cfu/mL and 6.74 cfu/mL	[7]
DNA	Magnetic particles	Intrinsic Peroxidase-Like Activity	20 nM	[8]
Single-nucleotide polymorphism	Streptavidin-coated magnetic beads	Intrinsic Peroxidase-Like Activity	0.4 fM	[9]
TP53 gene	Hemin/SWNT	Intrinsic Peroxidase-Like Activity	0.11 nM	[10]
SARS-CoV-2-spike protein	Magnetic nanoparticles/streptavidin-coated magnetic beads		84 fM	[14]
MicroRNA	Magnetic nanoparticle	Particle rolling circle amplification (RCA)	1 fM	[16]
MicroRNA	Magnetic nanoparticle	DNAzyme-assisted	1.5 pM	[17]
SARS-CoV-2	Magnetic beads/ Cas13	Colorimetry	200 fM	[18]
SARS-CoV-2 N gene/ monkeypox virus (MPXV) F3L gene	Cas 12a/DNAzyme	Chemiluminescent	1.5 and 2.4 pM	[19]
Single nucleotide polymorphism	Gold nanoparticles	Aggregation	5 fmole	[20]
Different ssDNA species	Graphene/ Gold nanoparticles	Intrinsic Peroxidase-Like Activity	11 nM	[21]
DNA	Gold nanoparticles	Colorimetry	-	[22]
Single-nucleotide polymorphism	gold nanoparticles	Aggregation	-	[23]
Single-nucleotide polymorphism	gold nanorods gold nano-triangles	Aggregation	-	[24]
Multiplexed	Gold nanoparticles	Colorimetry	5 nM	[25]
Cancer cells	Magnetic beads	Colorimetry	4 cells	[26]
MERS, MTB, HPV	Silver nanoparticles	Lateral flow assay	1.53 nM. 1.27 nM. 1.03 nM	[27]

Single-nucleotide polymorphism	Silver nanoparticles	Colorimetry	11 nM	[28]
Cancerous exosome	Biotinylated aptamers	Colorimetry	7.7×10^3 particle/mL	[29]
SARS-CoV-2-spike protein	Quantum dots	Colorimetry	NA	[30]
Sickle cell anemia	Copper nanoparticles	-	0.64 nM	[31]
SARS-CoV-2-spike protein	Gold nanoparticles	-	48 ng/mL	[32]
SARS-CoV-2-spike protein	Magnetic nanoparticles	Colorimetry	4.98 ng/mL	[33]
SARS-CoV-2 R and E gene	Gold nanoparticles / Magnetic beads	Colorimetry	R gene: 10 nM E gene: 20 nM R+E= 500 pM	[34]
SARS-CoV-2 variants	Urease (LFA)	Visual/ pH indicators		[35]
Salmonella typhimurium	Gold on gold-tip/ RNase H2	Fluorescence	3.2×10^3 CFU mL−1	[36]
SARS-CoV-2 variants	Nanobeads	Colorimetry	100 ng/mL	[37]
SARS-CoV-2	Rolling circle amplification (RCA)/ Streptavidin-coated magnetic beads	Colorimetry	0.2 pM	[38]
SARS-CoV-2 nucleocapsid	Fe_3O_4-AgMBA@Au nanoparticles	LFA/SERS (Lateral flow assay/ surface enhanced Raman spectroscopy)	10^{-8} mg/mL/ 0.08 pg/mL	[38]
miRNA-155	Magnetic beads/ copper nanoparticles	Intrinsic Peroxidase-Like Activity	22 aM	[39]
Ochratoxin A	hemin-encapsulated and copper nanoclusters (CuNCs) functionalized DNA hydrogel	Peroxidase activity of DNAzyme/ Fluorescence	0.25 ng/mL / 3.49 pg/mL	[40]
Sarafloxacin	Fe/Co-Metal organic framework@Pt NPs	Intrinsic Peroxidase-Like Activity/ Fluorescence/ SERS	0.05 ng/mL / 0.125 ng/mL / 0.125 ng/mL	[41]

2. Protein Biomarkers

Researchers use colorimetric techniques to detect proteins through nanoparticle-based methods or by enhancing traditional assays like ELISA (Enzyme-linked immunosorbent assay) with nanomaterials. For example, scientists have detected dopamine and lysozyme by aggregating functionalized gold nanoparticles [42, 43]. Similarly, they have used magnetic particles with intrinsic peroxidase-like [44] activity to identify the SARS-CoV-2 spike protein.

A modified ELISA approach employs cesium lead iodide nanocrystals ($CsPbI_3$ NCs) coated with phospholipids ($CsPbI_3$ NCs@PL) to visually detect nuclear matrix protein 22 (NMP22), a marker for bladder cancer. This

method shows sensitivity on par with commercial kits and offers excellent recovery and specificity [45]. In cardiovascular diagnostics, researchers used peptide-functionalized gold nanoparticles to detect cardiac troponin in 200-fold diluted rabbit serum. This method completed detection within 10 minutes and reached a detection limit of 0.1 ng/mL [46]. Another study used DNA tetrahedron nanostructures (DTNs) with platinum nanoparticles (PtNPs) to design signal probes for detecting cardiac troponin, myoglobin, and C-reactive protein. These probes provided tunable valency and delivered a 94.7% recovery rate in 10% serum samples [47]. Researchers enhanced nanomaterials and integrated biotin-streptavidin interactions to boost sensitivity, increasing assay selectivity and lowering detection limits.

Another team developed a CRISPR/Cas12a-based immunoassay to detect the SARS-CoV-2 nucleocapsid (N) protein with ultra-sensitivity. They used a capture antibody to isolate the N protein, followed by sequential binding with a secondary antibody (Ab1), a biotinylated antibody (biotin-Ab2), and streptavidin, which anchored a biotinylated primer. This method achieved a detection limit of 1 fg/mL [48]. Likewise, using Cas14 with isothermal amplification, scientists also detected aflatoxin B1 in real samples with high specificity through a traditional colorimetric approach [49]. Modified nanomaterials, with their larger surface areas, provided customizable surfaces, better biocompatibility, and increased signal amplification.

Additional colorimetric immunoassay used Au@PtNPs conjugated antibodies to detect the SARS-CoV-2 N protein in untreated throat swab samples within 40 minutes at femtogram sensitivity [50]. Researchers also explored coordination polymeric nanomaterials (CPs), which offer strong chemical and thermal stability. One method used zinc-based CPs (ZnCPs) combined with alkaline phosphatase (ALP) and anti-carcinoembryonic antigen (anti-CEA) antibodies to detect markers in 50-fold diluted human serum [51]. Another distinct approach, Mehdi et al. created gelatin-coated gold nanoclusters (AuNCs) layered on gold nanoparticles to detect matrix metalloproteinase-9 (MMP-9), a cancer biomarker. When MMP-9 digested the gelatin layer, the restored fluorescence and AuNP aggregation produced a detectable purple color shift in saline [52].

Table 2. Colorimetric detection of various protein biomarkers

Analyte	Approach	Method	Limit of detection	Linear range	Ref.
SARS-CoV-2-spike protein	Colorimetry	Gold nanoparticles	48 ng/mL	NA	[32]
Histamine	Colorimetry	Aggregation	1.57 µM	1–100 µM	[42]
Lysozyme	Colorimetry	Gold nanoparticles based	16 nM	10 -100 nM	[43]
SARS-CoV-2-spike protein	Colorimetry	Intrinsic Peroxidase-Like Activity of magnetic particles	4.98 ng/mL	NA	[44]
Urinary nuclear matrix protein 22 (NMP22)	Colorimetry	Sandwich assay	0.03 U/mL		[45]
Cradiac Troponin	Colorimetry	Aggregation	8.4 pM	16.8 pM – 84 pM	[46]
Multiplexed (Cardiac Troponin, Myoglobin, c-reactive protein)	SPR (Surface Plasmon resonance)	Platinum nanoparticles assisted signal probes	Troponin – 3 pM Myoglobin- 0.4 nM CRP- 6.7 nM	NA	[47]

SARS-CoV-2 Nucleocapsid protein	ELISA	Sandwich assay	1 fg/mL	3 fg/mL – 3 x 107 fg/mL	[48]
Aflatoxin B1 (AFB1)	Colorimetry	Cas14a-assisted nanoprobe composite of iron and gold	31.90 pg/mL	0.05 to 10 ng/mL	[49]
SARS-CoV-2 Nucleocapsid protein	Colorimetry	Au@Pt NPs enabled microfluidic device	0.1 pg/mL	NA	[50]
Carcinoembryonic antigen	Colorimetry	Coordination polymeric based nanoparticle assisted sandwich immunoassay	21.1 pg/mL	0.05 to 100 ng/mL	[51]
Matrix metalloproteinase (MMPs)	Fluorescence	Gelatinized gold nanoparticles aggregation	2 ng/mL	10–100 ng/mL	[52]
SARS-CoV-2 spike protein	ELISA	Sandwich assay	16.8 ng/mL	1-100 ng /mL	[53]
Lectin	Hydrogel	Photonic crystals	0.3 nM	0.3 nM – 4.23 nM	[54]
Multiplexed (Orchatoxin A, zearalenone, deoxynivalenol, and aflatoxin B1)	Colorimetry	-	0.06, 0.72, 11.89, and 0.04 µg/kg	NA	[55]
C-reactive protein	Colorimetry	Hollow ruthenium nanoparticles (HRNs)	33.2 pg/mL	0.12–7.8 ng/mL	[56]
Multiplexed (Tumor necrosis factor alpha (TNF-α), Interferon gamma (IFN-γ) and Interleukin-2 (IL-2)	Colorimetry	Microfluidic based	TNFα- 0.6 ng/mL IFN-γ- 0.34 ng/mL and IL-2- 0.56 ng/mL	1 -100 ng/mL	[57]
SARS-CoV-2 spike protein	Lateral flow assay	Immunoassay based on core-shell nanoparticles	1.22 pg/mL	102pg/mL to 106 pg/mL	[58]

3. Future of Diagnostics: AI in Healthcare Diagnostics

Traditional biosensing methods often rely on predefined threshold values or manual interpretation of signal outputs, which can be prone to variability, human error, and limited sensitivity in complex or low-concentration samples [59]. As diagnostic targets become more diverse and sample matrices become more heterogeneous, conventional analytical approaches struggle to capture subtle patterns or nonlinear relationships in the data [60]. Machine learning (ML) and artificial intelligence (AI) offer powerful tools to address these limitations by identifying intricate signal features, learning from large datasets, and enabling data-driven decision-making [61].

These tools typically follow a common machine learning pipeline. First, a dataset is constructed from either raw sensor outputs—such as absorbance spectra, fluorescence intensity changes, or RGB colorimetric responses—

or from captured images of assay results. The data then undergoes pre-processing, which may include denoising, normalization, standardization, background correction, and in some cases, augmentation to improve generalizability. The data must be accurately labelled for supervised learning tasks according to the target classes (e.g., disease states, analyte types, or concentrations). The dataset is then divided into training, testing, and sometimes validation subsets to evaluate model performance and prevent overfitting. Finally, the processed data is input into one or more ML/AI algorithms. A variety of models have been used across different applications, including support vector machines (SVMs), random forests (RFs), k-nearest neighbours (KNNs), and convolutional neural networks (CNNs), with model selection often tailored to the nature of the input data and the complexity of the classification task.

Yang et al. developed a machine learning-assisted colorimetric sensor array using Fe single-atom nanozymes functionalized with four ligands to detect over 10 UTI-related microorganisms. UV-vis absorbance changes from each sensor were used to create feature vectors, reduced to 2D using U-MAP. A linear Support Vector Machine (SVM) was trained on this data to classify healthy, bacterial, and fungal infections, achieving up to 97–100% accuracy in clinical urine samples [62].

Du et al. created a fluorescence/colorimetric dual-mode sensor array using small-molecule probes to detect and classify five amyloid fibrils. Fluorescence intensity and smartphone-derived RGB features were processed with linear discriminant analysis (LDA), principal component analysis (PCA), and hierarchical cluster analysis (HCA). LDA was the primary supervised ML tool used for classification and quantification. PCA and HCA were used for visual analysis to validate the LDA results [63].

Wei et al. introduced a DNA-AuNP-based colorimetric sensor array to classify 12 proteins by measuring salt-induced aggregation via absorbance ratios (K/K_0). Using LDA and HCA, they achieved 100% classification accuracy in both standard and human serum samples, demonstrating high sensitivity and robustness with just two nonspecific DNA probes [64].

Draz et al. developed a smartphone-based CNN system to detect ZIKV, HBV, and HCV by analysing bubble patterns generated from platinum nanoparticle-catalyzed reactions. Using transfer learning on Inception v3 which is a pre-trained CNN, the CNN achieved up to 98.97% sensitivity and functioned without optical attachments, making it suitable for low-cost, point-of-care diagnostics [65].

Davis & Tomitaka used CNNs and traditional ML algorithms (Random Forest, SVM) to quantify SARS-CoV-2 N-protein levels from smartphone images of lateral flow assays. By training on augmented, multi-resolution images, CNNs achieved up to 95.8% accuracy, outperforming other models in noisy or color-varied conditions. Random Forests were more efficient on smaller, low-resolution inputs [66].

Xue et al. created a smartphone-based deep learning system for interpreting CRISPR-Cas13 LFA results using U-Net and mobile-optimized MnUV3 segmentation models, followed by CNN classification. Trained on 3146 annotated images, the models achieved 96.5% accuracy, enabling real-time, low-cost COVID-19 testing without hardware constraints [67].

Materón et al. used a smartphone-based plasmonic biosensor and machine learning to detect SARS-CoV-2 in saliva and river water. RGB data and absorbance spectra were classified using a Random Forest algorithm, achieving 100% accuracy in differentiating infected and healthy samples. This low-cost, portable method is ideal for point-of-care diagnosis [68].

Lee et al. introduced SMARTAI-LFA, a smartphone-based COVID-19 diagnostic platform using deep learning with YOLOv3 for test line detection and ResNet-18/50 for classification and quantification. Trained on over 16,000 LFA images, it achieved up to 98.7% accuracy, outperforming human experts and enabling real-time, cradle-free, point-of-care testing [69].

Shen et al. developed a 12-unit fluorescent sensor array optimized to 3 key probes for urinary protein detection. Fluorescence response vectors were classified using LDA and HCA, achieving 100% accuracy in identifying individual proteins, mixtures, and disease-specific urine samples, demonstrating strong diagnostic potential [70].

4. Conclusion

Early-generation biosensors primarily relied on simple mechanisms such as nanoparticle aggregation or enzyme-mimetic catalytic activity for analyte detection. However, recent advancements have led to the incorporation of highly sensitive amplification techniques—including rolling circle amplification (RCA), duplex-specific nuclease (DSN)–mediated signal enhancement, and hybridization chain reaction (HCR)—which enable the detection of biomolecules at ultralow concentrations, even in complex biological matrices like serum, plasma, or saliva. The integration of functional nanomaterials, particularly magnetic nanoparticles and engineered nanostructures, has further enhanced biosensor performance by facilitating automated signal processing, target enrichment, and miniaturization, making these platforms highly adaptable for point-of-care diagnostic applications.

In parallel, the convergence of biosensing technologies with artificial intelligence (AI) and machine learning (ML) has addressed several inherent limitations of traditional diagnostic platforms, such as signal subjectivity, low reproducibility, and limited multiplexing capability. As the field continues to evolve, the combination of advanced materials, miniaturized hardware, and data-driven algorithms is expected to further enhance the functionality and accessibility of biosensors. Ultimately, these developments pave the way for more personalized, decentralized, and preventive healthcare solutions, with wide-ranging applications across clinical, environmental, and public health domains.

Conflicts of Interest

The authors declare that there is no conflict of interest for this article.

Authors' Contributions

All authors made equal contributions.

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