Nanoparticle-based Point of Care Immunoassays for in vitro Biomedical Diagnostics

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In resource-limited settings, the availability of medical practitioners and early diagnostic facilities are inadequate relative to the population size and disease burden. To address cost and delayed time issues in diagnostics, strip-based immunoassays, e.g. dipstick, lateral flow assay (LFA) and microfluidic paper-based analytical devices (microPADs), have emerged as promising alternatives to conventional diagnostic approaches. These assays rely on chromogenic agents to detect disease biomarkers. However, limited specificity and sensitivity have motivated scientists to improve the efficiency of these assays by conjugating chromogenic agents with nanoparticles for enhanced qualitative and quantitative output. Various nanomaterials, which include metallic, magnetic and luminescent nanoparticles, are being used in the fabrication of biosensors to detect and quantify biomolecules and disease biomarkers. This review discusses some of the principles and applications of such nanoparticle-based point of care biosensors in biomedical diagnosis.

Keywords Nanoparticles, nanomaterials, diagnosis, paper-based assays, microPAD, point of care

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1 Introduction

The alarmingly high prevalence of communicable and non-communicable human diseases has made real time diagnosis indispensible for prompt treatment. Timely diagnosis can undoubtedly improve clinical outcomes, such as progression of disease, irreversible disease side effects and temporary/lifelong disability. To improve on these diagnostic limitations, scientists are pursuing major biomedical research initiatives to adopt the latest technologies. Traditional in-vitro healthcare diagnostic methods, such as enzyme-linked immunosorbent assay (ELISA),
radioimmunoassay (RIA), high-performance liquid chromatography (HPLC), real-time polymerase chain reaction (RT-PCR), quantitative polymerase chain reaction (qPCR), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), though fundamental to the diagnostic field, have associated cost and time constraints. Scientists are continuously introducing innovations to the field of diagnostics to make it real time and cost effective. One focus of this reframing is to provide healthcare facilities at the patient’s door, i.e. point of care (POC) diagnostics. These POC diagnostics, which include lateral flow assays, dipstick assay and paper-based microfluidic analytical assays, are used for qualitative and quantitative monitoring of analytes and have been effectively developed for clinical chemistry, immunology, hematology, microbiology and molecular diagnostics.1–8

Nanotechnology is the scientific discipline to manipulate atoms and molecules to produce nanostructures with at least one dimension smaller than 100 nm. Nanotechnology is benefited by the fact that by reducing the size of materials to the micro/nano level, the surface area of the metal increases, which results in its enhanced reactivity. At this micro level, the optical, magnetic and electrical properties of the structures become highly controllable by varying the size and shape. Nanoparticles of different chemistry, size and shape have been synthesized, and are being used widely in various sectors like agriculture, textile, energy, health and biotechnology.

Different technologies are being used or under development for POC diagnostic assays, which are easy to use, highly sensitive for low-limit detection and cost effective.9 Nanotechnology, a most innovative and attractive technology, has the potential to be used for portable POC applications. Currently, researchers are working to incorporate nanomaterials in POC diagnostic assays to introduce new innovations to develop diagnostic assays that offer the advantages of low cost, greater sensitivity, requirement for fewer reagents, and being contamination free. Incorporation of nanotechnology in the fabrication of POC biosensors has resulted in enhanced sensitivity and specificity of assays and miniaturization of cost-effective diagnostic assays, systems and devices as compared to their conventional techniques. Moreover, it is hoped that use of nanotechnology will enhance the diversity and scope of biomedical diagnostic applications.10–12

POC diagnostic assays are generally performed on a small chip usually made of silicon, paper, glass or polymer materials having micrometer scale channels. These channels are partitioned into testing zone and sample zone. These channels are used to divert the biological sample to the testing zone where the mixing and chemical reaction of the sample with reagents take place to detect the required analytes. The detection is based on some chemical or enzymatic reactions that generate different colors depending upon the analytes, e.g. protein and glucose. The color change depends upon the soluble organic dyes being used in the assay. In the development of these types of immunoassays, efforts are being made to improve the detection limit, as sometimes the detection limit is so low that the color change cannot be discriminated by the naked eye. This situation brings challenges when there is a need to use the assay to detect the concentration of relevant analytes to check the type of disease, e.g. metabolic disorders, different types of renal disorders depending upon the concentration of protein.13–16

To address the diagnostics hurdles, and to enhance the sensitivity of the assay, nanoparticles-based immunoassays have been reported, which rely on the unique properties of nanoparticles and have improved detection limit up to 1000 times.17–20

2 Nanostructures and Nanoparticles

The unique tunable physicochemical properties of nanomaterials (e.g. size, chemistry, etc.) have made them suitable for applications in designing highly sensitive diagnostic assays. Depending upon the nature of the immunoassays, nanoparticles are used either directly as chromophore agents or the surface of nanoparticles can be modified by attaching molecules, e.g. antibody, peptide or DNA fragment, to detect the target protein

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Fig. 1  Mode of action of nanoparticles. Nanoparticles (NPs) are used either directly as chromophore agents (a) (adapted from Ref. 81) or the surface of NPs is modified by attaching molecules, e.g. DNA fragment or antibody to detect the target DNA sequence (b) or protein (c).
or DNA sequence. This reaction takes place in a microfluidic system, which provides the platform to regulate the flow of the sample through micro-channels (Fig. 1). Different nanoparticles, such as gold, silver, copper, ceria and magnetic nanoparticles like iron oxide or quantum dots, are being used in in-vitro diagnostic assays to provide low limit detection and quantification of biomolecules like glucose or protein concentration. A comparison of size and activity of various nanoparticles used in different diagnostic biomedical applications is provided in Table 1.

| Classification of NPs | Type of nanoparticles | Size/nm | Activity | Reference |
|-----------------------|-----------------------|---------|----------|-----------|
| Metallic Au           | 13                    | —       | 36, 37   |           |
| Au                    | 60                    | —       | 38       |           |
| Au                    | 13                    | 0.1 nM  | 39       |           |
| Au                    | 13                    | 0.25 nM | 89       |           |
| Ag                    | 20 – 80               | 30 pg/mL| 59       |           |
| Ag                    | —                     | 0.25 mg/dL| 53    |           |
| Ag                    | 22                    | 1.5 µg/mL| 60     |           |
| CuO                   | —                     | 0.1 nM  | 62       |           |
| CuO                   | —                     | 3.0 x 10^(-4) ng/mL, 6.1 x 10^4 U/mL, 2.9 x 10^(-4) U/mL, 1.4 x 10^(-4) ng/mL, for AffP, CA 125, CA 153 and CEA | 63 | |
| Pt-Pb                 | —                     | 0.05 ng/mL| 64     |           |
| Magnetic FeO₄         | 50 – 20000            | 0.066 fmol| 32     |           |
| Magnetic              | 50                    | 1 pg/mL and 100 fg/mL for HNE and Cathepsin-G | 67 | |
| FeO₄                  | 153.6                 | 1 ng/mL | 75       |           |
| Silica                |                       | 0.1 µM  | 70       |           |
| Silica                | 290                   | —       | 78       |           |
| Silica                | 50                    | 250 ng/mL| 79     |           |
| Ceria                 | 20 – 100              | 0.5 nM  | 81       |           |
| Luminescent Quantum dots | 247                | 75 pg/mL| 86       |           |
| Luminescent Quantum dots | 40                 | 1 ng/mL | 87       |           |

2-1 Metallic nanoparticles

2-1-1 Gold nanoparticles as a signal transducer (fluorophore)

In recent years, the visual detection of change in chromogenic behavior of gold nanoparticles (AuNPs) has supported their position as the most promising fluorophore in different biomedical diagnostic assays like biosensors and colorimetric biosassays (e.g. lateral flow assays). The characteristic fluorescence behavior of different-sized AuNPs and their ability to easily couple with biological material have revolutionized the colorimetric biosassays, especially paper-based diagnostic assays. AuNPs, either as a single chromophore or coupled with other chromogenic agents, significantly enhance the limit of detection, sensitivity and specificity of the paper-based assays. One key feature that makes them the most ideal candidate as a chromogenic agent for developing bioassays is their color change (fluorescence) due to aggregation of AuNPs. When small sized AuNPs, usually 2 - 40 nm in diameter, come in close contact, they form aggregates due to surface plasmon absorption that results in intense color change and this distinctive characteristic makes the AuNPs good candidates for use as colorimetric probes/labels in immunoassays. Other distinct properties that make AuNPs highly tunable include their higher extinction coefficient, controllable surface chemistry, easy conjugation to biomolecules and versatility. AuNPs have been used to detect and screen many analytes, including DNA, protein, enzymes, hormones, ions and cancer biomarkers.

The distinct optical properties of AuNPs have been coupled with the catalytic amplification of the traditional enzymatic chromogenic agent horseradish peroxidase (HRP) to develop a dual label (i.e. HRP-AuNP) assay for lateral flow nucleic acid biosensors (LFNAB) for hybridization-based ultrasensitive naked eye detection of DNA (Figs. 2(a) and 2(b)). For LFNAB, AuNPs were synthesized by tetrachloroauric acid (HAuCl₄) reduction with trisodium citrate and the HRP-AuNPs complex was prepared by the subsequent addition of thiolated detection DNA probe and HRP in AuNPs (~15 nm) solution. It has been reported that sodium dodecyl sulfate (SDS) (added to increase the stability of AuNPs), thiolated DNA and HRP played a vital role in improving the detection limit of this sensor up to 0.01 pM of the target DNA.

Sequence specific DNA identification has proved to be a decisive biomarker in designing simple, specific and sensitive assays for the diagnosis of different diseases. Instead of laborious laboratory procedures, AuNPs have been conjugated with functional polymers (biopolymer or artificial) to achieve improved sensing properties. A variety of biosensors have been designed based on different aggregation systems of DNA-AuNPs conjugates, i.e. cross-linking and non-cross-linking methods. In one study, AuNPs (13 nm) were synthesized by citrate reduction method and were then functionalized by thiol-modified oligonucleotides from different Mycobacterium tuberculosis complex (MTC) members. The objective was to identify the causative agent of human and animal tuberculosis, i.e. MTC, and differentiate M. bovis and M. tuberculosis using the conserved gene sequence of gyrB locus as target. In this Au nanoprobe-based molecular detection assay, the colorimetric identification was used on the principle of hybridization of PCR amplified gyrB gene from each strain and species specific Au-nanoprobes.

To avoid time-consuming steps associated with the purification and analysis of amplicons (PCR amplified target sequences), like washing and incubation and use of thiol-modified AuNPs, microfluidic paper diagnostic assay employs unmodified AuNPs (13 nm), synthesized by citrate reduction of HAuCl₄, for rapid identification of single stranded target DNA sequence (24-mer) of tuberculosis mycobacterium, responsible for tuberculosis (TB). Colorimetric sensing of this TB diagnostic assay is based on salt (NaCl) triggered aggregation of AuNPs with analyte solution having detection oligonucleotide sequence and extracted DNA sequence from patient or control subject.

Gold nanoparticles (60 nm) coupled with 35-mer thiolated oligonucleotides have been used as a probe in the lateral flow test to detect amplified human immunodeficiency virus (HIV) RNA concentration quantitatively (Fig. 2(c)). Lateral flow assays based on AuNPs have been developed for human HIV nucleic acid detection and myoglobin (MYO) detection. For human HIV nucleic acid detection, AuNPs (diameter of ~13 nm) were modified with HIV detector probe, while for MYO detection, MYO antibody was used to modify AuNPs (diameter of ~30 nm). Single-stranded DNA detection and quantification by using hepatitis B virus (HBV) as a model has been achieved by designing AuNP-based biosensor. Similarly, AuNP-based lateral flow strips have been designed for quantitative analysis of human immunodeficiency virus type 1 (HIV-1) DNA.

AuNPs (42.7 nm) employed in LFA have been effectively used for the diagnosis of different diseases. Instead of laborious laboratory procedures, AuNPs have been conjugated with functional polymers (biopolymer or artificial) to achieve improved sensing properties. A variety of biosensors have been designed based on different aggregation systems of DNA-AuNPs conjugates, i.e. cross-linking and non-cross-linking methods. In one study, AuNPs (13 nm) were synthesized by citrate reduction method and were then functionalized by thiol-modified oligonucleotides from different Mycobacterium tuberculosis complex (MTC) members. The objective was to identify the causative agent of human and animal tuberculosis, i.e. MTC, and differentiate M. bovis and M. tuberculosis using the conserved gene sequence of gyrB locus as target. In this Au nanoprobe-based molecular detection assay, the colorimetric identification was used on the principle of hybridization of PCR amplified gyrB gene from each strain and species specific Au-nanoprobes.

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to diagnose HBV infection by detecting HBV surface antigen (HBsAg) as a biomarker. In another study, LFA based on AuNPs have been developed to detect phospholipase-A2 (PLA2) in human serum.

AuNPs synthesized by successive ionic layer absorption and reaction (SILAR) approach were directly deposited on a paper-stripe to develop a label-free biosensing strip for early diagnosis of keratoconjunctivitis, an infectious eye disease. In another study, a AuNP-based immunosensor was developed for the detection of malaria using Plasmodium falciparum histidine-rich protein 2 (PfHRP 2) as a biomarker (Fig. 2(d)).

AuNP-based disposable DNA biosensor strips have also been reported for quick and easy detection of food borne pathogens such as Vibrio cholerae. AuNPs (40 nm) coupled with anti-fluorescein antibodies were employed to form a detector reagent in a glass fiber-based DNA biosensor for the detection of biotin and fluorescein labeled PCR amplified target. The shelf life of these DNA biosensor strips was 30 days at 37°C.

AuNPs have also been used to monitor dihydronicotinamide adenine dinucleotide (NADH) concentration in different NAD-driven enzymatic reactions. Detection of anxiety biomarkers, i.e., cortisol hormone, has been made possible by employing AuNPs in aptamer-based biosensors. AuNPs (15 nm) synthesized by citrate reduction method have been bioconjugated with antibodies to develop an immunochromatographic strip test for rapid identification of the typhoid causing agent, Salmonella typhi (S. typhi) in human serum (Fig. 2(e)).

2-1-2 Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) have also been used in biomedical diagnostic assays; their applications relative to AuNPs are limited due to certain associated issues such as lack of size homogeneity during synthesis, less intense color in solution and vulnerability to oxidation. Due to surface plasmon resonance, AgNPs exhibit a color change from yellow to red/brown due to aggregation.

AgNPs have been employed either in unmodified form or in conjugation with other metallic nanoparticles. In one study, silver deposition, which is catalyzed by gold nanoparticles, has been used in paper-based diagnostic assays for malaria to intensify the colorimetric detection of the malarial infection biomarker Plasmodium falciparum histidine-rich protein 2. Paper-based analytical devices for cholesterol detection have been coupled with a silver nanoparticle modified boron-doped diamond (AgNP/BDD) electrode to improve the analytical capability of the device. In another study, unmodified Ag nanoprobe were used to detect enzymatic reactions in biologically complex systems in real situations.

In another study, a label-free bimetallic (core of AgNPs and shell of AuNPs) cellulose substrate-based biosensing strip was designed to monitor the progression of invasive breast cancer in urine samples. Modified AgNPs have been used to design a
label-free colorimetric assay to detect neurogenin 1 (ngn1) protein, involved in the differentiation of neurons (Fig. 3(a)). 50 Label-free AgNPs have been studied for colorimetric detection of cysteine (Fig. 3(b)) and trypsin amino acids (Fig. 3(c)). 56–58 In-vitro detection of protein biomarkers, such as α-fetoprotein (AFP) and C-reactive protein (CRP), has been studied by designing AgNP-based fluorescence detection systems. 59 Label-free AgNPs have been explored for their application in designing colorimetric methods for detection of entecavir, an antiviral agent used in the treatment of hepatitis B virus infection. 60

2·1·3 Copper oxide nanoparticles

Copper oxide nanoparticles (CuO NPs) are also being used as fluorophore to develop immunoassays. The chemical composition of CuO NPs is Cu 79.87% and oxygen 20.10%. In these assays, CuO NPs showed high sensitivity and results were observable by the naked eye. This copper assay has been successively used to diagnose HIV. 27 In another study, CuO nanoparticles were utilized to label antibody used for H1N1 influenza virus detection. 61 In one study, poly thymine (poly T)-templated CuNPs were employed as signal reported probes in a

Fig. 3 Silver nanoparticle based assays. Schematic representation of neurogenin 1 (ngn1) detection using AgNPs (a) (adapted from Ref. 50), in which mercaptosuccinic acid (MSA) functionalized AgNPs have been used to detect ngn1 using anti-ngn1 antibody. The addition of only NaClO₄ salt results in the aggregation of AgNPs and a color change from yellow to red is observed, while adding both salt and ngn1 results in no aggregation and no color change. Detection principle for cysteine (b) and trypsin (c) using AgNPs.

Fig. 4 Schematic representation of paper-based immuno-device for tumor marker identification using CuO nanoparticles.
fluorescent biosensor to detect interaction of protein (streptavidin) with small molecules (biotin). In order to overcome the limitations associated with screening of cancer biomarkers, paper-based immunogenic assays have been designed for cancer biomarker screening in which CuO NPs have been used to label the secondary antibody (Fig. 4). Platinum-palladium (Pt-Pd) bimetal nanoparticles

Pt-Pd bimetal nanoparticles have been used in paper-based strips to detect the p53 tumor suppressive protein. This lateral flow assay provides enhanced visual and quantitative detection of protein due to strong peroxidase-like activity of Pt-Pd NPs. This bimetal approach is extendable to other protein biomarkers.

2-2 Magnetic nanoparticles (MNPs)

Gold nanoparticles are being used as highly chromophore agents due to their high optical activity and chemical stability; however, these are prone to circumstantial factors. So other nanomaterials like magnetic nanomaterials (MNPs) are being explored as bio-label material for their applications in different bioassays. Due to their high stability and reproducibly, these are extensively used as sensing elements in different bioassays. Carboxy group modified magnetic nanoparticles (MNPs, 200 nm) based lateral flow immunoassay (LFIA) has been developed for the detection of carcinoembryonic antigen (CEA), a significant biomarker for detection of different types of cancers including breast cancer, lung cancer and bladder cancer. In another study, magnetic nanoparticles were incorporated on an optogenetic sensor for detection of C-reactive protein (CRP), an inflammatory biomarker.

In another study, a colorimetric assay based on magnetic nanoparticles (50 nm) was developed for the detection of inflammatory salivary biomarkers, i.e. human neutrophil elastase (HNE) and cathepsin-G to detect periodontitis. Gold magnetic nanoparticles (GoldMag) have been used to develop a point of care genotyping assay, i.e. detection of single nucleotide polymorphisms (SNP). In this lateral flow assay genotyping of methylenetetrahydrofolate reductase (MTHFR) C677T was studied as an example, which is extendable to other genes. Superparamagnetic nanoparticles (SPMNPs, ∼15 nm) with carboxyl groups, prepared by one-step hydrothermal synthesis process, have been used to develop quantitative lateral flow immunoassays (LFIA) for detection of carbohydrate antigen 72-4 (CA72-4) which is a gastric cancer biomarker, in human serum (Fig. 5(a)).

2-2-1 Iron oxide (Fe3O4)

Iron oxide (Fe3O4) magnetic nanoparticles consist of a magnetic core surrounded by a non-magnetic shell. Their size ranges from 50 nm to 20 μm. These are important in developing biomedical applications such as DNA isolation, separation and immunoassays owing to their large surface-to-volume ratio. Due to this property, they have increased analyte capture efficiency. Magnetic microparticles have been used to detect and quantify hepatitis C virus core protein antigen in blood and the target biomarkers of tuberculosis. These have also been used to develop immunoassays to detect causative agents from...
Iron oxide nanoworms have also been used to develop synthetic biomarkers to monitor urinary disease. These synthetic biomarkers constitute substrate-reporter peptides conjugated with carrier iron oxide nanoworms (Figs. 5(b) and 5(c)). MicroPAD treated with Fe₃O₄ nanoparticles have been used in colorimetric detection of glucose concentration. This Fe₃O₄ nanoparticle-based treatment created not only a biocompatible and highly catalytic surface on paper-based substrate but also solved the color homogeneity problem, which heavily affected the detection limit of the assay. Fe₃O₄ nanoparticles (153.6 nm) fabricated through the hydrodermal polyol process have been applied in immunochromatographic assay for the sensitive and specific detection of hepatitis B surface antigen (HBsAg). Before conjugation of Fe₃O₄ nanoparticles with anti-HBsAg antibodies, they were treated with polyacrylic acid for carboxyl surface modification (Fig. 5(d)).

Silica nanoparticles as a tracing tag to label signal antibodies

One of the major drawbacks associated with the use of traditional chromogenic agents to develop color contrast in paper-based assays is heterogeneity of the color distribution in the test zone. To overcome the problem of color uniformity, intensity and sample wicking velocity, silica nanoparticles have been used in microPADs by capturing them within the three dimensional structure of cellulose fibers. This device was successfully used to perform bioassays for the qualitative and quantitative analysis of lactate, glucose, and glutamate. In order to overcome drawbacks associated with previously used ocular infection detection devices/assays, anti-acanthamoeba antibodies coupled with fluorescent silica nanoparticles (290 nm) have been used in fluorescent immunochromatographic assay (FICGA) for prompt detection of acanthamoeba antigens and hence diagnosis of acanthamoeba keratitis, a rare ocular infection that could lead to blindness (Fig. 5(e)).

Silica nanoparticles doped with cyanine-5 (Cy5), the most commonly used fluorescent dye in biological assays, has been reported for use in the development of fluorescence-based lateral flow immunoassay (LFIA) for influenza A antigen detection. Carboxyl-modified silica nanoparticles (50 nm), prepared by Stober method, were linked to the amino group of monoclonal antibody specific to influenza A nucleoprotein through carbodiimide chemistry. The sensitivity of this assay was eight times higher than gold nanoparticle-based LFAs.

2-2-3 Ceria nanoparticles as quantitative chromogenic indicator

Apart from inter-particle distance as in the case of AuNPs, changes in other physicochemical properties of nanoparticles can also be used as indicators in colorimetric bioassays. Ceria or cerium oxide (CeO₂) nanoparticles could be used as a chromogenic agent in developing colorimetric bioassays due to their antioxidant activity at physiological pH values. In such assays, the color change of ceria particles is dependent on the change in the redox state of nanoparticles on the addition of the analyte. In response to the analyte, the redox state and composition of nanoparticles change and result in clear visible color change that could be seen by the naked eye. This concept has been applied to detect glucose concentrations in human serum by using different sized nanoparticles, i.e. 20 and 100 nm.
Smaller sized nanoparticles (20 nm) showed an intense orange/brown color while larger sized nanoparticles (100 nm) showed a minor change in color. Hence, surface-to-volume ratio of the nanoparticles is an important feature to be used in designing such assays. The shelf life of this bioassay was observed to be up to 79 days at room temperature.\(^{81}\)

### 2.3 Luminescent nanomaterials

In spite of the fact that gold nanoparticles have been applied to a number of applications, the current studies toward investigating new nano-chromophore materials have identified that some luminescent nanomaterials have even better sensitivity and detection limits than gold nanoparticles. Traditionally used gold nanomaterials in different chromogenic test strips are weaker due to their low detection limit. Luminescent nanomaterials such as quantum dots offer high sensitivity.

Strong absorption and narrow emission spectrum bands of luminescent nanomaterials, namely quantum dots (QDs), have made them an established candidate for coupling with paper-based substrates to enhance the detection limit and hence analytical performance of the common paper-based analytical assays.\(^{52-54}\) Among different luminescent nanomaterials, QDs have various applications in the field of biomedical diagnostics, especially in the detection of cancer biomarkers in blood.\(^{85}\)

Ultra-chromogenic signal amplification has been achieved by employing quantum dot bead (QB) probes in immunochromographic assay (ICA) for the highly sensitive, qualitative and quantitative detection of hepatitis B virus surface antigen (HBsAg) in human serum. Synthesis of QB probes involves covalent coupling of the amino group of anti-HBsAg monoclonal with the carbonyl group of carboxyl-modified QBs (247 nm), prepared by encapsulating CdSe/ZnS using a micro-emulsion method.\(^{86}\)

A similar approach has been applied in another study where a sandwich immunoassay-based immunochromatography test strip (ICTS) was developed, which incorporated CdSe/CdS quantum dots (QDs, 40 nm) as luminescent label to detect alpha fetoprotein (AFP), a cancer biomarker in human serum (Figs. 6(a) and 6(b)).\(^{87}\)

QDs have been used to develop paper-based solid-phase nucleic acid hybridization assays in which QDs (donor) were immobilized on modified surface of paper, which in turn were paired with cy3 (acceptor)-labeled oligonucleotide target (Fig. 6(c)).\(^{88}\)

### 3 Conclusion

Use of nanoparticles in designing in-vitro diagnostic assays has resulted in ultra-high sensitivity and specificity. Interestingly, bioassays based on conjugation of chromogenic agents like horseradish peroxidase (HRP) or potassium iodide with nanoparticles have even greater signal amplification of up to 100 fold better than traditional chromogenic dyes used alone. It is hoped that these assays will serve as a guide to explore other nanomaterials that are even more cost effective and have enhanced fluorescent properties in designing bioassays, which could be used to detect other disease biomarkers. Undoubtedly, such systems have great potential to change the landscape of disease diagnostics and treatment, which will eventually contribute to the reduction of preventable deaths.

### 4 Conflict of Interest

All authors have no conflict of interest with regard to this article.

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