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Chapter

# Application of Quantum Dots in Lateral Flow Immunoassays: Non-Communicable and Communicable Diseases

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## Abstract

Quantum Dots (QDs) are ideal fluorescent labels for lateral flow assays (LFA) due to their unique optical properties and resistance to chemical and photo-degradation. Lateral flow assay, known as immunochromatography test, is a diagnostic strip test that uses paper or membrane-based devices to detect the presence/absence of an analyte with the pregnancy test, the most known LFA. Diagnostic tools for non-communicable (NCDs) and communicable diseases (CDs) are available. However, other NCDs and CDs do not have LFAs as first-line diagnosis/point-of-care. QDs are promising fluorescent labels as they improve the LFA's colourimetric sensitivity and fluorescence stability. This chapter discusses the tailoring and application of QDs in LFA. Particular focus on the applications of QDs in LFA for detecting or screening NCDs (i.e. cancers etc.) and CDs (i.e. SARS-CoV-2, HIV/AIDs etc.).The book chapter concludes by discussing different challenges and perspectives of QDs in LFA.

**Keywords:** quantum dots, lateral flow immunoassays, point-of-care, communicable disease, non-communicable disease

## 1. Introduction

Non-communicable diseases (NCDs) (commonly known as chronic diseases) are diseases that result from environmental, behavioral and genetic reasons. As a result, they tend to linger for extended periods. Some NCDs like cancer, diabetes, and heart disease are preventable through lifestyle and behavior changes [1]. In comparison, communicable diseases (CDs) are diseases that can spread from one person to another (i.e. via bodily fluids and blood, inhaling airborne virus) [2]. Early detection and rapid diagnosis of NCDs and CDs are essential for disease screening and treatment. Several diagnostic tools are available for the detection and prognosis of NCDs. These include: biopsy procedures (i.e. endoscopic, pelvic examination, bronchoscopy, pap test and lumbar puncture.) [1], blood tests (i.e. complete blood count, blood protein testing etc.) [3] and diagnostic tests (i.e. computerized tomography (CT) scan and

magnetic resonance imaging (MRI) scans) [4]. Nonetheless, these medical tools are costly, laborious, and require clinical sample preparation and sophisticated instruments with trained operators.

Moreover, they are unsuitable for first-line diagnosis. This has resulted in a need to develop cost-effective, rapid, and reliable methods for clinical diagnoses and prognoses of NCDs and CDs. Point-of-care testing (POCT) tools are medical diagnostic tools used at the time and place of patient care [5]. It employs simple equipment and reduces the time needed to generate clinical results, permitting patients and clinicians to make on-the-spot clinical decisions. POCTs have some advantages over traditional diagnostic tools, i.e. shorter detection time, less costly, limited to no sample processing, basic instrumentation, with minimal operation requirements [6]. Therefore, they permit early detection of diseases, especially in resource-limited areas, thereby improving the time of initial treatment. Nonetheless, the unavailability of clinical POCTs for NCDs such as cancer and the emergency of the COVID-19 pandemic has highlighted the need for developing POCT tools to allow for improved tracking and response time.

Lateral flow assay (LFA) (also known as immunochromatography tests) is one type of POC testing tool that has addressed the challenges of traditional testing streams like the polymerase chain reaction (PCR) and reverse transcription-polymerase Chain Reaction (RT-PCR). The most common LFA example is the pregnancy and human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) test. LFA is a simple paper or membrane-based device that uses a liquid sample to detect the presence or absence of the test analyte (or disease biomarker). LFA has several advantages as an attractive sensing tool because it is portable, low cost, and enables on-site detection (**Table 1**) [7]. In LFA, various materials have been employed to detect the presence/absence of a test analyte in a liquid sample. The use of nanomaterials in the development of LFA has enabled qualitative naked-eye detection, broadened the type of labels used, eased antibody/antigen conjugation, and improved fluorescent and dual colourimetric signal detection [8]. Several nanoparticles used in LFA include magnetic nanoparticles [9] and colored nanoparticles [10], with fluorescent quantum dots serving as promising fluorescent probes. Quantum dots (QDs) are ideal fluorescent labels for immunoassay application. Their unique optical properties, molecular extinction coefficients, and excellent resistance to chemical and photo-degradation have advantaged them significantly over fluoro-dyes [11]. In LFA application, QDs have been used in antigen-antibody reactions to detect a variety of

| Advantages |
|---|
| • Low Cost. |
| • Simple and user-friendly operation. |
| • Stability in different environments/ settings |
| • Has long shelf life. |
| • Less time for analysis. |
| • Very little to no energy consumption. |
| • High potential for commercialization. |
| • Changeable formats, bio-label molecules, and detection zones. |

**Table 1.**
*Advantages of lateral flow assay (LFA).*

analytes, i.e. tumor/biomarkers [12, 13], bacteria [14], and viruses [15]. Bock et al., 2021 [16] developed a QDs-embedded with silica nanoparticles lateral flow immunoassay to detect prostate-specific antigen (PSA). The study used QDs as a probe for their red-emitting properties. The group coated the test line with anti-PSA antibody and the control line with goat anti-mouse IgG antibody. Bovine serum albumin (BSA), sucrose, Poly(ethylene glycol) (PEG), Tween 20 and QDs-PSA were used to condition the conjugate pad of the LFA. The LFA results were visible within 15 min, with an LOD of 0.138 ng/mL and no false-negative results on the clinical samples.

Moreover, the fluorescence intensity of the strip exhibited no significant decrease for 10 days. Detecting low levels of antibodies at the early stages of diseases has been a significant challenge in clinical diagnosis. Currently used early-stage diagnosis methods are still expensive with long turn-around times. Kim et al., 2022 [17] reported on the highly sensitive LFA kit based on brush-type ligand-coated quantum beads (B-type QBs) with the potential to diagnose several diseases by changing the antibody pair in the LFA kit. The B-type QBs were obtained via the self-assembly of polystyrene-co-poly(acrylic acid) (PS-PAA)and the QDs. The B-type QBs enabled good dispersion and stability and had a high-binding capacity towards antibodies. Controlling the orientation of the antibody created an ultra-high sensitive LFA. This study highlights the effect of antibody orientation and ligand type on LFA performance. Moreover, the human serum spiked hCG detection confirmed the LFA potential application in human body fluid.

This chapter addresses the application of lateral flow assays as a diagnostic tool for communicable and non-communicable diseases. The chapter discusses the basic principle and mechanisms of lateral flow assays. This is followed by discussions on the tailoring and application of quantum dots (QDs) in LFA to detect NCDs and CDs. The chapter then focuses on applying QDs in the LFA detection of various non-communicable and communicable diseases. The chapter further addresses some of the challenges within LFA application and concludes with future perspectives on QDs application in LFA.

## 2. Principle of lateral flow immunoassays

Lateral flow assays (LFA) are fabricated on a series of capillary beds made of a specific material. The material used (i.e. glass fiber or nitrocellulose membrane) contributes to the LFA performance. It ensures that the samples migrate towards the detector bed, which hosts the test analyte. The central beds of the LFA are (i) sample application bed, (ii) conjugate pad, (iii) substrate membrane, and (iv) absorbent bed (**Figure 1**).

### 2.1 Mechanism of lateral flow assay

The sample is presented at the sample bed (made from cellulose fiber) and migrates to the conjugate pad, which consists of material labeled with analyte-specific antibodies. The conjugate antibodies bind and move up with the sample towards the test line. The test and control lines (also known as the detection compromise of nitrocellulose membrane immobilized with biological moieties (primarily antibodies or antigens). When flowing into the detection zone, the conjugated sample reacts with the analyte bound to a specific antibody. Upon detection, visual changes appear as a line (for a positive test result), while the control line appears whether the target analyte is present or not [6, 7]. The conjugation of the label materials (i.e. quantum dots) to biomolecules such as antibodies allows for the improvement in the capture

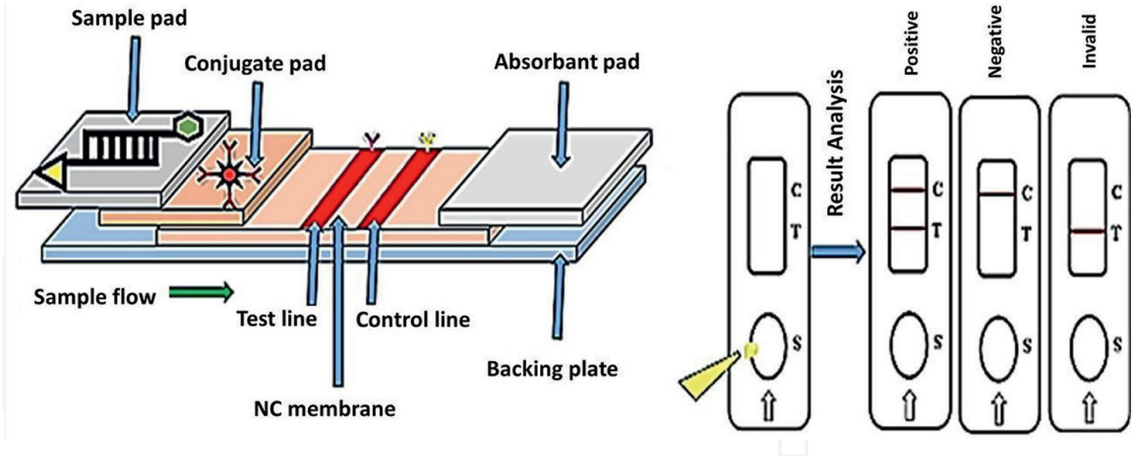


**Figure 1.**
*Schematic illustration of the lateral flow assay (LFA) . Reproduced from Huo C, Li D, Hu Z, Li G, Hu Y and Sun H (2021) a novel lateral flow assay for rapid and sensitive nucleic acid detection of Avibacterium paragallinarum. Front. Vet. Sci. 8:738558 under license https://creativecommons.org/licenses/by/4.0/ [18].*

and release of the target analyte in the LFA beds (majorly in the conjugate, test, and control beds). This improves the detection of disease biomarkers.

Although LFAs enjoy the above advantages, traditional lateral flow immunoassays do suffer from limitations which include but are not limited to:

i. Lack the adequate sensitivity that enables color changes that the human eye can view,

ii. Loss of conformation and recognition functionality in non-aqueous media by antibody biosensors,

iii. Challenges in the maintenance of colloidal and fluorescence stability in aqueous biological environments, particularly in enzyme-mediated conditions,

iv. Challenges of ligand exchange and

v. Qualitative results that suffer from obstruction of pores due to matrix components.

To address these challenges, optimization of LFA using various experimental approaches has been reported. Optimizing multiple parameters on the bed is necessary for optimal LFA performance enabling adequate flow, release, and stability. **Table 2** lists the components (i.e. buffers, stabilizers, detergents/surfactants/wetting agents and blocking agents) commonly used to optimize the efficiency, reproducibility, sensitivity, and minimize non-specific binding of the LFA. To further address some of the above LFA shortcomings, research has shifted towards applying QDs as signal-generating material (SGM) [17] (Kim et al., 2022). For example, Liang et al., 2020 [19] reported on a QDs-based LFA used in conjunction with a portable fluorescence immunoassay chip for detecting exact IgE for mite allergens obtained from patients with allergic rhinitis. Clinical samples were analyzed with good reproducibility, fluorescent test, and control line.

The brightness of QDs has therefore improved LFA sensitivity and *in-situ* monitoring [20]. Moreover, QDs have become essential in LFA due to their ease of bio-functionalization, which enables specific and stable recognition of antibodies,

| Component | Commonly used reagent |
|---|---|
| *Buffers*<br>• for ionic strength and pH<br>• reproducibility<br>• sensitivity and specificity | • Tris(hydroxymethyl)aminomethane (Tris)<br>• Glycine<br>• 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES)<br>• Bicarbonate<br>• Borate<br>• Phosphate |
| *Stabilizers*<br>• stabilizes capture bioreceptor | • Trehalose<br>• Sucrose<br>• Lactose |
| *Detergents/ Surfactants/ Wetting Agents*<br>• prevent non-specific binding<br>• Assist in the flow of detection labels along the LFA | • Polyoxyethylene sorbitan monolaurate (Tween)<br>• Sodium dodecyl sulfate (SDS)<br>• 3-[(3-cholamidopropyl)dimethylammonio]propane--1-sulfonate (CHAPS)<br>• Polyvinyl alcohol (PVA)<br>• Polyethylene glycol (PEG)<br>• Sodium deoxycholate |
| *Blocking agents*<br>• prevent non-specific binding<br>• improve specificity<br>• faster flow | • PEG<br>• Bis(trimethylsilyl)acetamide (BSA)<br>• Casein<br>• Milk<br>• PVA<br>• Chaotropic agents |

**Table 2.**
*Optimization parameters.*

addressing the lack of commonly reported conformation. QDs as fluorescent probes have thus become useful for the immune recognition of target analytes [21] (Wilkins et al., 2018), and allow for quantitative detection of biomarkers. In a study by Rong et al., 2021 [22], red-colored quantum dot nanobeads (i.e. silica or polymer nanobeads embedded with hundreds of quantum dots) were used for the multiplex and simultaneous detection of four infectious disease biomarkers. The group were able to detect human immunodeficiency virus (HIV), *Treponema pallidum* (TP), hepatitis C virus (HCV), and hepatitis B virus (HBV) at LOD levels of 0.11 NCU/mL, 0.62 IU/L, 0.14 NCU/mL and 0.22 IU/mL respectively within 20 min. The direct aqueous synthesis of QDs has further eliminated the challenges of colloidal and fluorescence stability of LFA when biomolecule (i.e. blood, serum, plasma etc.) samples are analyzed. While the small sizes and good size distribution of QDs prevents the obstruction/blocking of membrane pores in the sample pad and detection zone.

## 3. Engineering quantum dots for lateral flow immunoassays

Quantum dots are semiconductor materials with shape and size-dependent optical properties. They have found increased application in the diagnosis and prognosis of

infectious diseases [23]. QDs conjugated to biomolecules have improved the binding efficiency within the conjugate pad. QDs bonded to bio-recognition antibodies offer a viable system to enhance specificity and sensitivity in the detection zone. Optimizing multiple parameters for adequate flow, release, and stability is necessary for optimal LFA performance.

Most importantly, the label material must be able to detect antibodies to capture and release the test analyte sufficiently. Antibodies are used as bio-recognition components in LFA's detection zone (test and control lines), and they co-join the target analyte via immunochemical exchange [6, 24]. It is, therefore, essential that the QDs used are also able to bind with the conjugate pad material. The conjugate pad plays an essential role in running the LFA. It serves as the location for chemicals and ensures the constant transfer of detector reagents and analytes to the detector zone. It is, therefore, necessary to ensure QDs exhibit non-specific binding to conjugate pad material to ensure sufficient target analytes reach the detection zones. The conjugate pad also houses the detector particles and ensures they are kept functionally stable until the test is used [8, 9, 25]. In instances where the QDs as fluorescent probes are not well coated or unstable, thus losing their properties over time may result in insufficient target analytes reaching the test line, reducing LFA sensitivity and signal intensity. Furthermore, delays in QDs bound to an analyte of interest due to extended interaction with conjugate pad material may cause inconsistent flow. This may result in the test and control lines appearing as fluorescent streaks due to inconsistent flow [9, 25].

To improve the binding efficiency of QDs, conjugation of QDs via carboxyl-reactive crosslinker reactive groups is commonly used for the labelling/crosslinking of QDs. Water-soluble 1-ethyl-3-(−3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) is used for aqueous crosslinking in conjunction with *N*-hydroxy succinimide (NHS). NHS is commonly added to EDC coupling to improve its efficiency and stability. For non-aqueous organic synthetic methods, N′, N′-Cyclohexyl carbodiimide (DCC) crosslinker is widely used. Carbodiimide crosslinkers function by activating the -COOH functional group of the QDs, allowing for direct conjugation to primary amines ($–NH_2$) via amide bonds [26]. The functionalization of QDs in LFA enables increased sensitivity and specificity for a 2-part recognition scheme whereby the label and capture agent become specific to the target analyte [21].

QDs are often functionalized with different capping ligands, which provide colloid solubility (i.e. convert hydrophobic material to hydrophilic), inherent stability and assist in chemical binding for biomolecules (i.e. DNA, proteins, peptides, and enzymes) [27, 28]. **Figure 2** shows various generic QDs solubilization and biofunctionalization often performed to condition QDs. QDs synthesized using an organic medium are insoluble and require phase transfer via ligand exchange (**Figure 2a, b**) to improve their water solubility and enable their bio-application. Ligand exchange, therefore, converts the hydrophobic ligands with bifunctional (viz., drugs, antibodies, or proteins) moieties enabling direct bio-application of the QDs [28, 29]. **Figure 1c** shows the use of polymers to insulate the hydrophilic surface of the QDs. In contrast, **Figure 1** d-f and g depicts the aqueous dispersion and derivatization of the QDs surface and its interaction with amphilic 'diblock' and 'triblock' copolymers and phospholipids. **Figure 1h** shows the attachment of QDs with amino acids (protein or peptides) as a bio-functionalization technique, therefore solubilizing and converting the surface of the QDs.

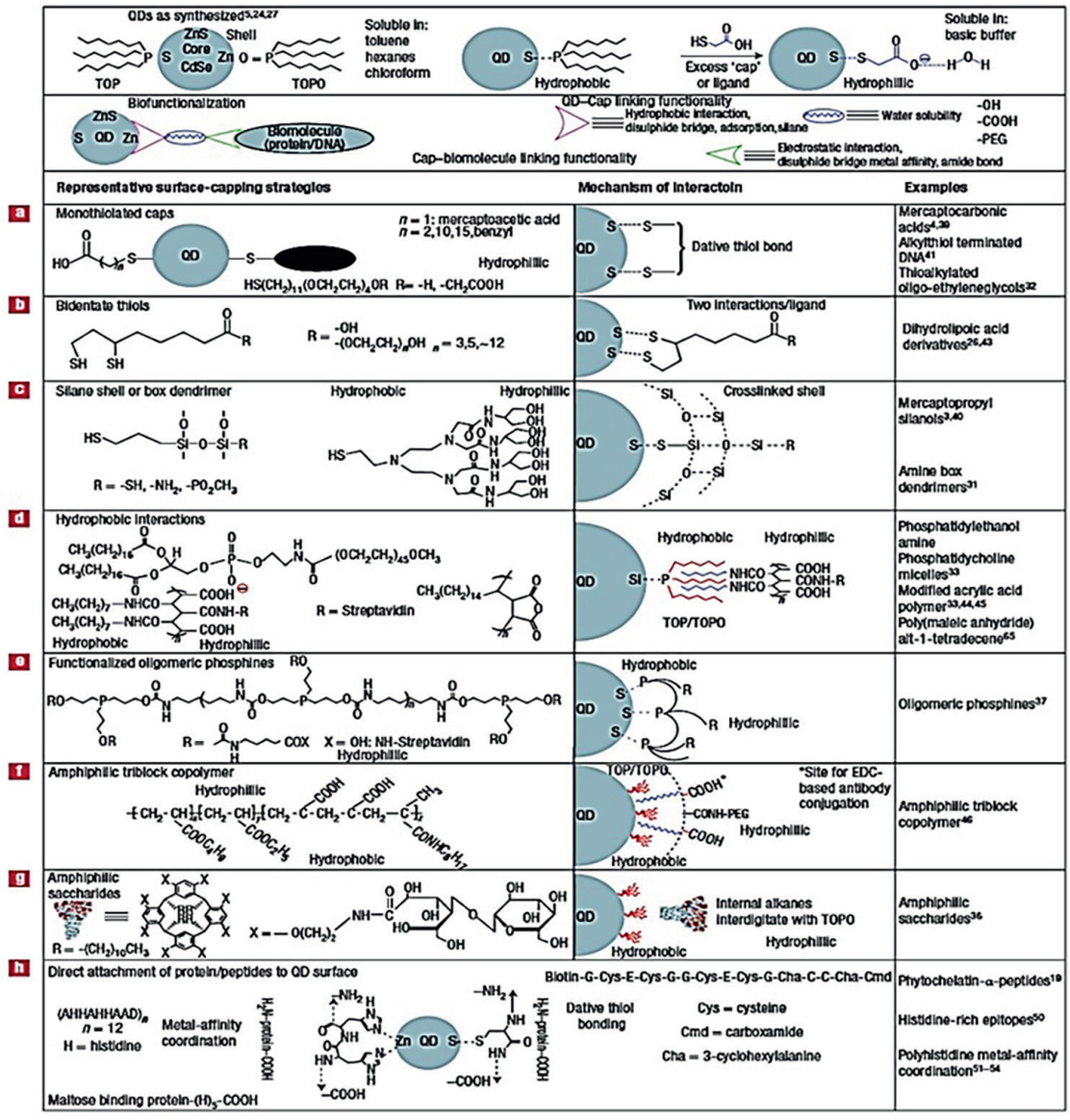


**Figure 2.**
*Schematic of generic QDs solubilization and biofunctionalization (a–h). Reprinted by permission from springer nature customer service Centre GmbH: Nature, nature materials IGor L. Medintz, H. Tetsuo Uyeda, Ellen R. Goldman and Hedi MattoussI), [COPYRIGHT] (2005) [28].*

## 4. Application of quantum dots in LFA to detect communicable (infectious) diseases

Quantum dots have superior fluorescent properties that render them ideal labels. Their application in LFA has improved the detection sensitivity of LFA as a result of their chemical-physical properties (i.e. high signal-to-noise ratio, narrow emission, high fluorescent quantum yields, and broad excitation) [30]. The bright luminescence and stability of QDs qualify their use in LFA for the sensitive detection of communicable (infectious diseases) and non-communicable (chronic) diseases.

### 4.1 Severe acute respiration syndrome coronavirus 2 (SARS-CoV-2)

Severe acute respiration syndrome coronavirus 2 (SARS-CoV-2) originated in 2019 as the source of the coronavirus disease 2019 (COVID-19). Real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) has been the primary test used for the molecular detection of SARS-CoV-2. Its application requires a certified laboratory infrastructure and trained personnel [31]. Nonetheless, for large-scale testing, quick results and contact tracing, RT-PCR is not a suitable diagnosis technique. COVID-19 diagnostic LFIA tests have provided fast, low-cost, and are a widely available tool for COVID-19 testing. Generally, these essential requirements are performed by serological tests based on lateral flow immunoassay (LFIA) [32]. The mechanism of COVID-19 detection/sensing using QDs is based on various paths that include; (i) inhibition of the binding of S protein receptors of the virus to the host cells, (ii) the generation of antiviral radicals upon exposure to light, (iii) reducing of viral RNA genome amplification and (iv) application of QDs as bright labels for COVID-19 [33, 34]. The unique properties (i.e. water solubility, small size ~10 nm, high sustainability, alluring photoluminescence) of carbon QDs (CQDs) have seen their application in the detection, inhibition, and treatment of viral infections [35]. Research on the interaction of QDs with genetic material (i.e. RNA and DNA), and cellular receptors to inhibit biological processes is still at its infancy. Nonetheless, their small sizes, ability to interact with cellular material, biocompatible nature and tuneable optical properties has seen growing reports on their importance in disease/virus inhibition processes. Loczechin et al., 2019 [33] reported on synthesizing carbon dots and their concentration-dependent interference with HCoV-229E-Luc infection. The mechanism of inhibition was said to be based on the CQD's ability to act in the early stages of virus infection by inhibiting the protein S-receptors with the host cell membrane. The group reported that the CQDs inhibited the HCoV-229E from entering the host cells infection as the functional groups of the CQDs interacted with the HCoV-229E entry receptors [36]. Another study by [19] reported on viral inhibition properties of CQDs via type 1 interferon responses. The study reported that the CQDs functioned by expressing IFN-stimulating genes (ISGs) and inducing interferon-α (IFN-α) production, which are responsible for inhibiting virus replication. Recently, Li et al., 2022 [36] reported on a smartphone-based QD-LFIA to detect IgG and Nab specific to SARS-CoV-2 in biological samples. The QDs were conjugated to anti-His mAb via covalent attachment (i.e. EDC/NHS). To enhance the performance of the LFIA, parameters such as the quantity of anti-His mAb-labeled QDs, the type of sample pads, the sample and the treatment buffer to reduce non-specific interactions within the QDs-LFIA were optimized. The developed QD-LFIA quantitatively detected IgG and Nab. The accurate and sensitive detection of the immune status of the clinical samples compared well with other methods. This highlights the progression in QD-LFIA application for the clinical detection of COVID-19.

### 4.2 Human immunodeficiency virus (HIV)

Human Immunodeficiency Virus (HIV) is an immunodeficiency disease that progresses into acquired immune deficiency syndrome (AIDS) at low CD4 levels (<200 cells/uL) [37, 38]. Currently, the most used approach to detect HIV is a dip-stick test which detects the virus antibodies [39]. The growing interest in the application of QDs for the detection of HIV is a result of QDs (i) small size that permits ease of flow over the strip, (ii) fluorescence lifetime and photostability, which enables QDs to be

signifiers for detection, (iii) a surface area-to-volume ratio that enables increasing binding sites and (iv) ability to combine with bio-molecules with ease as a result of their compatibility and stability [40, 41]. Deng et al., 2018 [41] used a fluorescent LFA strip to detect HIV-DNA quantitatively. The group used a one-step synthetic method to assemble the CdTe QDs with hairpin DNA as the reporter probe. At the same time, strand displacement amplification (SDA) worked as an amplification tool. The LFA consisted of a test and control zone on a nitrocellulose membrane immobilized with streptavidin in biotinylated t-DNA and cDNA, respectively. The developed LFA strip reproducibly detected HIV-DNA using CdTe-dsDNA (double-strand DNA) at a LOD of 0.76 pm.

### 4.3 Influenza

Influenza is seasonal endemic viruses that circulate yearly, causing mild to severe illnesses. Type A viruses such as (H1N1) and A (H3N2) are seasonal influenza caused by human infection. While Avian influenza viruses, A (H5N1) A(H5N6), A(H7N9), and A(H9N2), usually passed to domiciliary poultry leading to high disease surges resulting in life-threatening infections among humans [42]. Therefore, the accurate and fast diagnosis of influenza viruses is essential for early detection, hospital care and prevention of influenza infection outbreaks. The brightly fluorescent nature of QDs and bio-labelling properties have enabled the specific, accurate, quick, and simultaneous detection of *influenza* viruses. The sensitive, reproducible, and simultaneous detection of influenza A virus subtypes H5 and H9 using water-soluble COOH- functionalized QDs has been reported [43]. The QDs were covalently bounded to the influenza A virus antibodies and used as fluorescent tags in the LFA. The study harnessed the fluorescent signal produced by the QDs on the LFA strips to quantify the influenza A viruses (at LOD =0.016 HAU and 0.25 HAU for influenza virus H5 and H9 subtypes, respectively). In another study, Nguyen and co-workers [42] produced a CdSe/CdS/ZnS QDs LFA for sensitive and quick analysis of two influenza subtypes (H1N1 and H3N2). The test and control lines were coated with anti-influenza monoclonal and goat anti-mouse IgG antibodies. Although the number of positive samples was limited, the study could detect A/H1N1 64-folds higher than rapid diagnostic test (RDT; Standard Diagnostics BIOLINE Influenza A/B). These works highlight the potential of fluorescent QDs-based LFAs for clinical use in detecting influenza strains.

### 4.4 Hepatitis B

Hepatitis B virus (HBV) is a viral infection that can be transmitted through contact with infected body fluids (i.e. infected blood) [44, 45]. Hepatitis B surface antigens (HBsAg) and envelope antigens (HBeAg) are recognized clinical indicators used in the diagnosis of HBV [45]. Therefore, the accurate and rapid detection of HBsAg and HBeAg antigens is essential for the clinical diagnosis of HBV. Gold nanoparticles (GNP) coated with streptavidin (SA – GNP conjugate pad), biotin and antibody (Bio – GNP – Ab conjugate bed) have been employed to advance the sensitivity of LFA in the detection of HBV [46]. However, the expensive synthesis of GNPs, the dependence of the LFA on the localized surface plasmon resonance effect of GNPs [47] and their low sensitivity towards HBsAg have resulted in the exploration of QDs for HBV detection [48]. The ease in size tuning, biocompatibility and stable fluorescence has further advantaged QDs over other nanoparticles in LFA development. Several studies have reported the effect of GNPs size on the sensitivity

and reproducibility of LFA [45, 46, 49, 50]. For optimum performance of LFA and for enhanced LFA sensitivity, the narrow size distribution of GNPs has been proposed. Kim et al., 2016 [45] reported a reduction in signal intensity with increased GNP size. The group attributed the results to reduced conjugation efficiency of GNP at sizes >42.7 ± 0.8 nm. In another study, Chotithammakul et al., 2021 [49] reported the synthesis of single and mixed-size GNPs for LFA detection of Albumin. Their results showed mixed-sized GNP conjugates exhibited enhanced signals with higher stability and narrower resonance peaks than GNPs with 20 nm sizes. The results suggest that the combination of different sizes of GNP can affect their plasmon resonance. Moreover, in the study, the conjugated mixed-size GNPs - antibodies (anti-BSA) accumulated at the test spot, which improved localized surface plasmon transduction resulting in enhanced optical labelling in the LFA.

### 4.5 Tuberculosis

Tuberculosis (TB) is recorded as the deadliest infectious communicable disease caused by *Mycobacterium tuberculosis* and has become a significant public health problem worldwide. Experienced physicians use chest radiographs in clinical practice to detect TB [51, 52]. The clinical diagnosis at reduced time and low cost via LFA is still very underdeveloped for TB diagnosis. Detection of *mycobacterial free tuberculosis* proteins (fprA) using QDs conjugated to two monoclonal antibodies has been reported. The fluorescent QDs were conjugated to the antibodies via a biotin-streptavidin bridge. In this specific LFA, the antibodies were used as capture probes and immobilized onto the test lines of the nitrocellulose strip. The double-antibody sandwich LFA format allowed recombinant fprA protein detection at ~12.5 pg./μL. Compared to traditional immune-based testes (i.e. dot immunobinding assay and Elisa), the antibody labeled QDs LFIA improved the sensitivity, speed (results within 10 min) and simplicity [53]. In another study, Kabwe et al., 2022 [54] explored a paper-based lateral for detecting anti-mycolic acid antibodies. The group used mycolic acid (MA) coated CdSe QDs as a luminescent probe to detect anti-MA antibodies, biomarkers for tuberculosis. The study's results supported test strip-based immunoassays for TB at low-cost and optimum speed.

## 5. Application of quantum dots in LFA for detecting non-communicable (chronic) diseases

### 5.1 Cardiovascular diseases

Cardiovascular diseases (CVDs) are disorders related to the heart, such as ischemic heart disease, heart failure, stroke, cerebrovascular diseases, thrombosis, arrhythmia, and cardiomyopathies [55]. N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a diagnostic cardiac biomarker for acute heart failure. However, inadequate products are available in the market for the diagnostic quantification of NT-proBNP. Wilkins and co [21] recently reported on sandwich LFA for NT-pro-BNP. The group used monoclonal antibodies conjugated to QDs via reductive amination. The study showed that conjugating QDs to the antibodies using a site-specific reductive amination strategy exhibited a higher signal at all tested concentrations than LFAs run using EDC-conjugated antibodies. Along with other studies [56, 57], this work verified the potential use of QDs conjugated to CVDs antibodies as a viable means for risk assessment of human cardiovascular diseases.

### 5.2 Cancer

Cancer is one of the leading causes of death all over the world. An early cancer diagnosis is meaningful and helpful for extending the overall survival of patients. Despite developing highly sophisticated diagnostic tools, these are expensive, time-consuming, and require complex operations and skilled personnel. Moreover, they are not suitable for the first-line diagnosis. One of the early diagnostic approaches for cancer is to test tumor markers. Carbohydrate antigen 72–4 (CA72–4) is a biomarker linked with the diagnosis and prognosis of early stages of gastric cancer. A CdSe QD labeled sandwich LFA coupled with a charge-coupled device (CCD)-based reader has been reported to detect CA72–4 in clinical samples. Monoclonal CC49 and B72.3 antibodies are often used to detect CA72–4; therefore, to improve the sensitivity of LFA, CC49 was conjugated to the CdSe QDs. The QD-labeled CC49 exhibited narrower emissions with strong fluorescence signals than the QDs alone and proved to be a suitable sensor probe for CA72–4 detection. The QDs-based LFA could sensitively detect CA72–4 at 2 IU/mL levels within 10 min. Furthermore, compared to the Roche electrochemiluminescence assay, the developed strip method has shown excellent reproducibility and specificity [58]. Magnetic-quantum dot nanobeads (MQBs) are composite nanomaterials with distinctive fluorescent and magnetic properties. Antibody-conjugated MQBs are employable in recognizing the immune and can magnetically separate analytes under complex samples. Rong et al., 2019 [22] conjugated MQBS to free prostate-specific antigen (f-PSA) and complexed prostate-specific antigen (c-PSA) as fluorescent probes for prostate cancer. The conjugates were used for immune recognition and simultaneous detection of f-PSA and c-PSA analytes in a complex biological matrix. The developed sandwich LFA detected both antigens (-PSA and c-PSA) on a single test line with high sensitivity and specificity.

## 6. Current challenges

Current LFAs use label materials (in the conjugate pad) that are synthesized in the hydrophobic state, unstable, lack colourimetric fluorescence stability and are incompatible with the protein–particle binding. For example, using enzymes results in detection sensitivity that depends on an enzyme-substrate combination [59]. Colloidal carbon nanoparticles as labels suffer from the non-specificity and the presence of irregular shapes [59]. Although quantum dots seemingly addressed some of these issues. The use of binary QDs (composed of groups I-VI and VII) is of concern as they are toxic by their very nature due to the presence of heavy metals such as Cd and Pb. Moreover, the risk of toxic heavy metals leaking within the LFA is a significant concern, raising toxicity, health, and environmental concerns. Additionally, the synthesis of currently used QDs has been via organic reagent, thus requiring ligand exchange which compromises the optoelectrical properties of the QDs required for ideal LFA development. Another shortcoming of current LFAs is that they: (i) lack the adequate sensitivity that enables color changes that the human eye can view, (ii) loss of conformation and recognition functionality in non-aqueous media by antibody biosensors, and (iii) challenges in the maintenance of colloidal and fluorescence stability in aqueous biological environments, particularly in enzyme-mediated conditions, (iv) challenges of ligand exchange and (v) qualitative results of that suffer from obstruction of pores due to matrix components. To address these challenges, there is a need to develop multi-colored ternary and quaternary QDs-based LFA for the clinical detection of single and multi-disease/virus biomarkers.

## 7. Future perspective and conclusion

Quantum dots' unique optoelectrical properties have significantly advantaged them as ideal labels in LFA. Their high quantum yields, molecular extinction coefficients and excellent r chemical and photo-degradation further enable their broader application in LFA sensing and diagnosis. An ideal label for application in LFA includes colloidal stability under various conditions and temperatures, suitability for detection over large dynamic ranges, reproducibility, efficiency without losing biological and chemical integrity or activity, specific binding, and sensitivity. These are often achieved via bio-functionalization of QDs with biomolecules (i.e. DNA, protein, and peptide) which further enables the binding and capture of target antibodies within the conjugate pad. In the clinical diagnosis and treatment of communicable and non-communicable diseases, QDs' ability to enter cells, thus inhibiting virus replication, has been shown as a treatment strategy. However, the lack of fast, specific, reproducible, and stable diagnosis tools still hinders early disease detection. The development of QDs-based LFA (for NCDs and CDs) for clinical application has been reported in the literature. Their clinical use is, however, still not approved. Therefore, the exploration of clinical trials for QDs -based LFA as an alternative POC for NCDs and CDs is greatly needed.

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## Conflict of interest

"The authors declare no conflict of interest."

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