BIO DESIGN

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# Highly sensitive and selective *in vitro* diagnostics based on DNA probes and aptamers

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**Medical diagnosis is very important and essential for maintaining a healthy life. *In vitro* diagnostics have recently been a focus of scientists and researchers because they have a number of merits over other diagnostic methods. In particular, nucleic acid-based diagnostic methods are powerful and promising techniques. In this review, various types of nucleic acid-based *in vitro* diagnostics are introduced. These methods can be categorized into three groups according to their analytical approaches. In addition, aptamer-based diagnostic methods are covered in greater detail because aptamers are promising materials for diverse areas, not only as alternatives to antibodies but also as the core components of analytical equipment. It is expected that *in vitro* diagnostics based on DNA probes and aptamers will become a valuable platform encompassing all types of diseases.**

## INTRODUCTION

Medical diagnosis is the process of attempting to determine the presence of a disease or disorder. There are several methods that can be used in the diagnostic process, including differential diagnosis, pattern recognition, and diagnostic criteria (Rodriguez, 1997). These methods are mainly based on certain symptoms or signs related to diseases or disorders. Therefore, symptoms or signs have to be precisely obtained to determine or identify diseases. A diagnostic test is a type of medical procedure by which the symptoms or signs of patients' diseases can be obtained (Parquin and Audry, 2012). Such tests include utilizing nuclear medicine techniques, measuring blood sugar, taking a complete blood count, and monitoring electrocardiogram readings. These diagnostic tests can be classified into several groups, such as physical examinations, medical imaging, and *in vitro* diagnostics (Stankovic, 2007).

The term "*in vitro* diagnostics" refers to methods of performing diagnostic tests outside of a living body in an artificial environment, such as a hospital or laboratory. The expression "*in vitro*" comes from Latin, literally meaning "within glass," because, in the past, many *in vitro* diagnostic tests were conducted using glass vessels. Currently, *in vitro* diagnostics include much more than simple assays conducted in test tubes and glass dishes (Turner et al., 1999). *In vitro* diagnostics are used for large-scale screening and medical diagnosis, from confirming a pregnancy to checking for the presence of an infectious disease, such as malaria (Dati et al., 2004).

*In vitro* diagnostics are becoming increasingly familiar, as self-tests, such as pregnancy tests and blood glucose monitors, becomes more common, because they have a number of benefits (Kaleva et al., 2008). *In vitro* diagnostics help to guide clinical choices on what treatment will be the most suitable, ranging from measuring one's blood pressure to detecting genetic disorders. At an even earlier stage, *in vitro* diagnostics can be used to detect conditions even before signs of disease appear, often preventing a great deal of suffering and occasionally unnecessary treatment (Renz, 2013). In many cases, the early diagnosis produced by *in vitro* diagnostics can provide better long-term outcomes by emphasizing the need for medical treatment or changing one's lifestyle. In some cases, the information from *in vitro* diagnostics can help prevent untimely death. In addition, *in vitro* diagnostics play a significant role in public health programs, such as in population screening for infectious diseases.

Unlike other forms of medical technology, *in vitro* diagnostics never directly interact with a human body because their purpose is not to have a direct therapeutic effect, but to offer essential information on a patient's health status. Thus, the expertise of the healthcare professional using *in vitro* diagnostics is crucial to ensure correct decision making for patient treatment and care (Shimauchi, 1994). As the world's population ages and the burden of chronic diseases increases, *in vitro* diagnostics will become increasingly important. In addition, the interest of scientists in this area has gradually increased since 1990. As a result, publications in this area have increased in the past two decades (Gottlieb and Woodcock, 2006).

*In vitro* diagnostics can be classified into three main types: clinical laboratory tests, near-patient tests, and self-tests. Clinical

  

laboratory tests are medical tests that should be conducted under a specialist laboratory environment due to their highly complex procedures (Wians, 2009). For clinical laboratory tests, samples are sent to the specialist laboratory, and the physician who has requested the test can receive the results after the completion of the analytical process. Blood count, blood lipid tests, detection of biomarkers, measurement of enzymes, and quantification of electrolytes fall under clinical laboratory tests (Favaloro et al., 2013). Near-patient tests are performed by medical experts, such as doctors, nurses, and paramedics, because they can be carried out without a specialist laboratory or equipment. The user can easily obtain and understand the information about the patient in a short period of time, and they may utilize this information to provide insight for a diagnosis (Delaney et al., 1999). The other term for near-patient tests is point-of-care testing. Self-tests are fabricated to be conducted by the patients themselves. The patients themselves can easily interpret the information from their self-tests because these tests do not require the use of any specialist facility or equipment. Therefore, these tests use equipment that is small, portable, and simple to use, and their results are easy to interpret (Zeren and Oztekin, 2006). Most of these tests are commercially available and are becoming increasingly commonplace. As an example, blood sugar tests for managing diabetes and pregnancy test strips are frequently utilized.

## AMPLIFICATION-BASED *IN VITRO* DIAGNOSTICS

Immunoassays have been widely used in the diagnosis of diseases because antibodies have a strong binding affinity for their specific targets (Ward et al., 2004). Immunoassays provide sensitive and rapid results due to the strong catalysis of enzymes. In addition, immunoassay methods are relatively easy to use compared to other traditional methods, such as chromatography. Immunoassays, however, have several drawbacks, such as the high cost for the development and production of antibodies and the lack of binding ability for small molecules (Jayasena, 1999). Moreover, some immunoassays are weak for detecting sample contaminants, which may interfere with the accurate detection of the target biomarkers (Jayasena, 1999).

In nucleic acid-based *in vitro* diagnostics, specially designed nucleic acids are utilized as alternatives to antibodies for immunoassays. Nucleic acid-based *in vitro* diagnostics detect the presence of a specific biomarker, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or proteins, in bodily fluid through various methods (Weile and Knabbe, 2009). For example, in the case of viral diseases, a biomarker, which may be any one of these biomolecules, from the virus can be captured and detected by specific nucleic acids. For non-infectious diseases, one method by which they can be detected is by the interaction between a specific gene associated with the diseases and a nucleic acid (Palecek and Bartosik, 2012). Nucleic acid-based *in vitro* diagnostics have some advantages over immunoassays, such as lower costs for the development and production of nucleic acids and their thermal stability. Sometimes, however, diagnosis using nucleic acids may fail to detect a biomarker when infectious organisms hide in the host organs. Therefore, nucleic acid-based *in vitro* diagnostics are synergetic with other diagnostic methods, such as amplification, sequencing, and hybridization as well as specific binding.

Polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) is commonly used in biological studies and applications to amplify DNA or RNA that is reverse-transcribed to cDNA with specific primers (Bustin, 2002). In many cases, these amplification methods are utilized to obtain a large amount of DNA fragments of interest, but PCR or RT-PCR is also widely used to detect the presence of a pathogen or target gene in diagnostic applications (Ho et al., 2013). They allow for the sensitive detection of target DNA or RNA and requires a relatively short time to obtain the results. These methods, however, require a highly skilled expert and specialist laboratory equipment.

Commercial diagnostic kits are available for several diseases, such as leishmaniasis, tuberculosis, and human immunodeficiency virus (HIV). The SMART Leish PCR assay is a qualitative diagnostic real-time PCR test for the detection of the *Leishmania* species and the identification of *L. major* in skin lesion scrapings and punch biopsies from individuals suspected of having cutaneous leishmaniasis (Vasoo and Pritt, 2013). The Amplified *Mycobacterium tuberculosis* direct (MTD) test is a method for the detection of Mycobacterium tuberculosis complex rRNA based on a target-amplified nucleic acid probe test (Hazbon, 2004). Finally, the Abbott real-time HIV-1 assay is an assay for the quantitative detection of HIV type 1 (HIV-1) nucleic acids from human plasma and dried blood spots (Gueudin et al., 2007).

Loop-mediated isothermal amplification (LAMP) is a technique similar to PCR, but it is conducted at a single temperature, without an expensive thermal cycler, as represented in Figure 1 (Tomita et al., 2008). LAMP is more amenable to near-patient tests because of its simplified amplification process and low cost. In addition, LAMP has been known to be insensitive to analysis problems that are commonly associated with complex samples, including blood and urine (Tomita et al., 2008). Its primer design is relatively complicated, however, because LAMP requires four primers to amplify six regions of target DNA. Thus, it is more suitable to utilize LAMP in diagnostic applications than in molecular biology applications. A LAMP assay for the diagnosis of malaria was reported (Poon et al., 2006). In the study, blood samples were heated and tested by the LAMP assay to detect the 18S ribosomal RNA gene of *Plasmodium falciparum*. The sensitivity and specificity of the LAMP assay for malaria were 95 and 98%, respectively. This method was recently improved by Surabattula et al. through simple DNA extraction (Surabattula et al., 2013).

## SEQUENCING-BASED *IN VITRO* DIAGNOSTICS

DNA sequencing is the method of identifying the exact order of

  

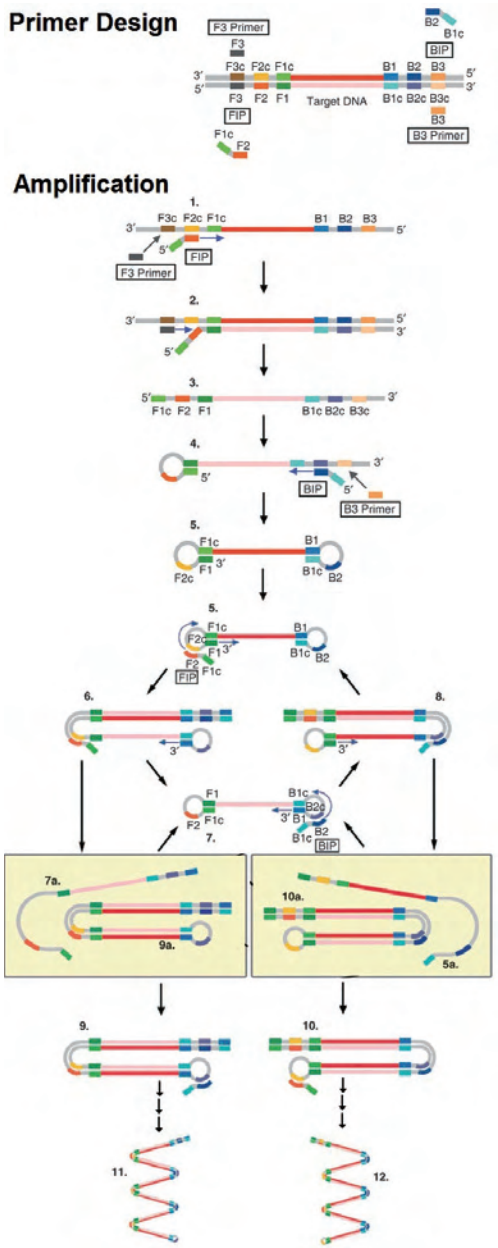


**FIGURE 1 | Principle of the LAMP method.** Six primer regions labeled F3, F2, F1, B1c, B2c, and B3c from the 5' end were designed (c: complementary sequence). Two inner primers, FIP (combination of F1c and F2) and BIP (combination of B1c and B2), and outer primers (F3 and B3) were utilized in this method. The template structure is synthesized from 1 to 5 step. The elongation is initiated via the hybridization between FIP and F2c primers (1). Strand displacement occurs through annealing of F3 primer to the F3c region (2), and then synthesized strand is released (3). That strand forms a loop structure at 5' end, and BIP and B3 primers are attached to that strand (4). DNA elongation proceeds in the same manner as described above. The resulting strand looks like dumbbell structure (5). This strand is amplified via cycling step (5-12). Self-primed DNA synthesis is started from the 3' end, and the elongation begins. Structure 7 that is complementary to structure 5 is produced through various steps, and structure 5 is regenerated from structure 8. Structures 9 and 10 are constructed from structures 6 and 9, respectively. Through the repetitive cycle, elongated strand is generated. The image is adapted from (Tomita et al., 2008).

nucleic acids in a DNA strand (Chan et al., 2013). Currently, it has been used for the identification of organisms in clinical fields, and various ribosomal genes, such as 16S and 23S, are used as the targets. DNA sequencing is routine and automatic via PCR and capillary electrophoresis, and the Sanger method is the most common method. Nevertheless, various sequencing techniques, such as single molecule real time (SMRT) sequencing, ion torrent sequencing, pyrosequencing, Illumina dye sequencing, and sequencing by oligonucleotide ligation and detection (SOLiD), have been developed to meet the high demand for low cost and high-throughput techniques (Meldrum et al., 2011). These sequencing methods are called next-generation sequencing and are very powerful and promising for the diagnosis of diseases. As shown in Figure 2A, SMRT sequencing uses the zero-mode waveguide (ZMW), an optical waveguide, and the fluorescence signal of each nucleotide is detected at the bottom of ZMW (Eid et al., 2009). It has 99.9% consensus accuracy and allows reads of 20,000 nucleic acids within two hours. SOLiD sequencing was

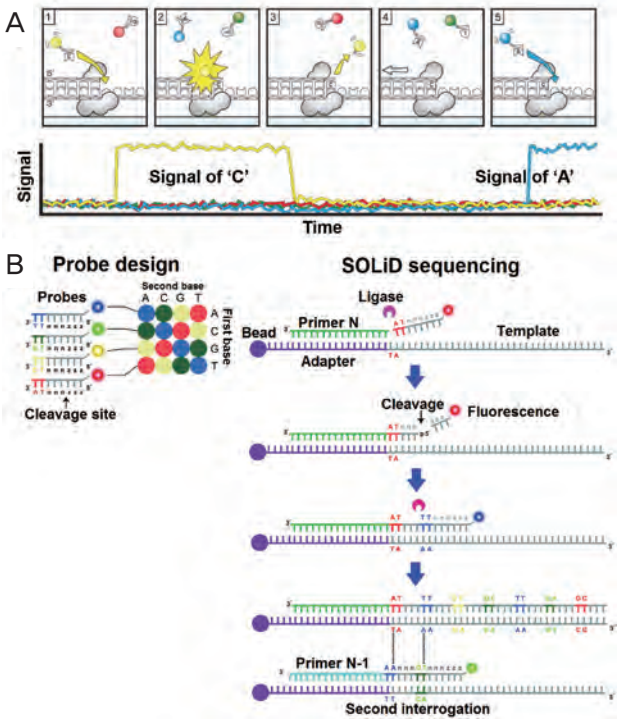


**FIGURE 2 | Hybridization and specific interaction-based *in vitro* diagnostics.** (A) Principle of the miRNA profiling microarray. The probe is immobilized on the detection plate and the target miRNA is labeled with the specific flourophore. And then, the target miRNA is hybridized with the probe, and the resulting signal is recorded. The image is adapted from (Li and Ruan, 2009). (B) Scheme for the detection of transcription factors using a specific DNA probe. Whereas the DNA probe is digested by exonuclease III in the absence of the target protein, the DNA probe-target protein complex is robust against to the digestion by nuclease in the presence of the target protein. Therefore, only DNA probe-target protein complex can exhibit FRET signal through the intercalation of Sybr Green I. The image is adapted from (Liu et al., 2013).

  

developed by Life Technologies in 2006 (Meldrum et al., 2011). It is based on a two-base encoding method and is performed through repeated oligonucleotide ligation and extension rather than through synthesis (Figure 2B). It has reduced the cost of sequencing to approximately US $0.0001 per base and has increased the capacity by 5 billion bases per day (Voelkerding et al., 2009). Likewise, the benefits of next-generation sequencing are considerable, but this process still has limitations, such as the requirement for expensive equipment, which could hinder its utilization in the diagnosis of diseases.

## HYBRIDIZATION AND SPECIFIC INTERACTION-BASED *IN VITRO* DIAGNOSTICS

*In vitro* diagnostic methods based on hybridization or binding are technologies for detecting the presence of biomarkers using a complementary DNA (cDNA) probe for target nucleic acids or specific sequences that bind to the target. Fluorescence *in situ* hybridization (FISH) is a typical diagnostic method based on hybridization (Gerami et al., 2009). It is utilized to localize the presence or absence of target DNA sequences in chromosomes. The cDNA probe is modified with fluorescent dye, and the locations of the cDNA probe on chromosomes are found using fluorescence microscopy. The role of the cytoplasmic tyrosine kinase JAK2 in human myeloproliferative disorder was revealed using this method (Baxter et al., 2005). Researchers found that there was a single point mutation in JAK2 in the patients in their study, and the mutation was identified using the FISH method. Another type of hybridization method is diagnosis using miRNAs, which are small non-coding RNAs containing approximately 22 nucleic acids (Cissell and Deo, 2009). miRNAs act as regulators in the transcription and post-transcription process, and the dysregulation of miRNAs is associated with various diseases, such as cancer, hearing loss, and growth defects. Thus, the detection of specific miRNAs is becoming increasingly important. Various detection methods for miRNAs have been developed beyond traditional Northern blotting, such as microarray technology (Figure 3A), a luminescence-based assay, and an electrochemical assay (Li and Ruan, 2009). These methods allow the sensitive, rapid, and multiplexed detection of the target miRNAs.

Various transcription factors, including miRNAs, perform important roles in the transcription process by binding specific DNA sequences; transcription factors can be associated with various diseases. Therefore, mutations in transcription factors or their presence at abnormal levels can be biomarkers for specific diseases. For example, mutation in insulin promoter factor-1 is associated with diabetes, and the STAT and HOX families are relevant to breast and prostate cancer, respectively (Libermann and Zerbini, 2006). In these cases, specific DNA sequences can be utilized to probe for these molecules due to their strong binding, which is similar to the antibody-antigen interaction that is vital for immunoassays. Therefore, it provides sensitive, rapid, accurate, and stable diagnostic techniques. One such detection

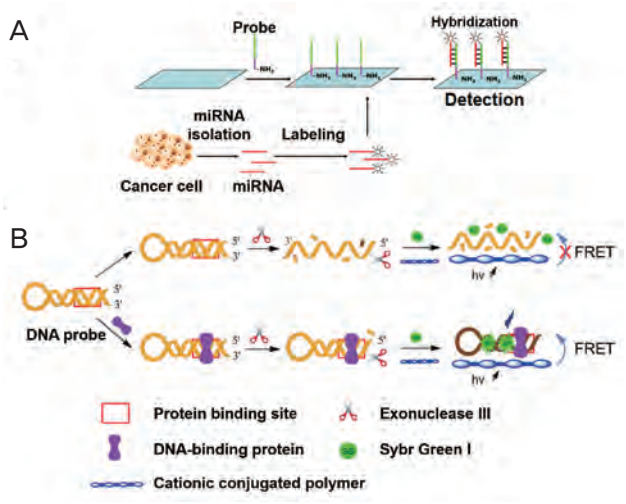


**FIGURE 3 | Sequencing-based *in vitro* diagnostics.** (A) Principle of SMRT sequencing. Fluorophore-conjugated nucleotide is incorporated in the strand, leading to an increase of fluorescence signal (1-2). Phosphodiester bond formation liberates the fluorophore, resulting that a decrease of fluorescence signal (3). This processes are repeated during SMRT sequencing (4-5). The image is adapted from (Eid et al., 2009). (B) Principle of SOLiD sequencing. In the probe design, each nucleic acid consisting of two specific nucleotides and six degenerate bases (nnnzzz) is conjugated with one of four fluorophores. In SOLiD sequencing, primer N is bound to the template, and two specific nucleotides complementary to the template are annealed and ligated. At this time, fluorescence is recorded, and then the fluorophore is cleaved. Through the repetition of these reactions, the first sequencing is carried out. For the complete sequencing, five additional sequencings are carried out utilizing successive primers (N-1, N-2, N-3, N-4, and N-5) in the same manner with the first sequencing. The image is adapted from (Voelkerding et al., 2009).

method for a transcription factor was recently reported (Liu et al., 2013). The specific DNA sequence containing the factor's binding site was used as a probe molecule, and the sensor system was designed using fluorescence resonance energy transfer (FRET) (Figure 3B).

## APTAMER-BASED *IN VITRO* DIAGNOSTICS

Aptamers are oligonucleotides or peptides that can strongly bind to specific targets via their specific three-dimensional structures. Aptamers are commonly created by an *in vitro* selection process called systematic evolution of ligands by exponential enrichment (SELEX) (Ellington and Szostak, 1990; Tuerk and Gold, 1990). At present, SELEX is a basic method for the discovery of aptamers, and specific aptamers can easily be selected *in vitro* against various targets, such as small molecules, biomolecules, and cells. In addition, aptamers have received great attention from many scientists because they have all of the advantages of antibodies, along with several unique advantages, such as thermal stability, low cost, low immunogenicity, variety of targets, and so on. Therefore, aptamers have been considered to be an alternative to antibodies in many biological studies and applications. Accordingly, aptamers have been studied

  

as biomaterials in numerous investigations concerning their use as a diagnostic and therapeutic tool in the development of biosensors, drugs, and drug delivery systems (Iliuk et al., 2011).

As mentioned above, SELEX is an *in vitro* selection technique for the identification of aptamers from approximately $10^{12}$–$10^{15}$ combinatorial oligonucleotide libraries consisting of random sequence regions, usually 40–50 mers, flanked by the primer binding site. The SELEX process generally consists of three steps, which are repeated to search for nucleotide sequences that can better bind to the target (Syed and Pervaiz, 2010). In the first step, the double-stranded nucleic acids in the library are converted into single-stranded nucleic acids. In the second step, the target-bound single-stranded nucleic acids are separated from unbound single-stranded nucleic acids. This step is commonly combined with other methods for the rapid selection of the target or library, such as nitrocellulose membrane filtration-based separation, affinity chromatography-based separation, and so on. In the last step, the target-bound single-stranded nucleic acids are amplified via PCR to generate a new library for use in the next round. Aptamers are discovered through this process, and their characteristics are identified via several biological assays.

There are various SELEX methods that are widely used to develop aptamers. Nitrocellulose membrane filtration-based SELEX was designed first (Tuerk and Gold, 1990). A nitrocellulose membrane is commonly employed to immobilize proteins in biological assays because it has a non-specific affinity for amino acids. A nitrocellulose membrane was utilized for the separation step in the early stage of SELEX because the targets at that time were primarily proteins. However, nitrocellulose membrane filtration-based SELEX has some limitations, such as its incapability to bind small molecules and peptides and the fact that it generally requires many selection rounds. Affinity chromatography is one of the best methods for the separation of biomolecules from a mixture. It has been widely used for the purification of recombinant proteins via a specific interaction between the target proteins and beads. In the binding and separation process of SELEX, the library components that possess affinity for the target can easily be selected by immobilizing target molecules on beads, which can be used to immobilize both proteins and small molecules (Song et al., 2011). Thus, both small molecules and proteins can be utilized for SELEX. The disadvantage of this method, however, is that it requires an additional affinity tag or special functional group to be added to the target molecules. Functionalized magnetic beads are also utilized to immobilize the proteins or small molecules via an electrostatic interaction or a covalent bond between the affinity tag and the substrate on the beads. The magnetic bead-based SELEX method recently attracted attention because magnetic beads are an especially powerful tool due to the easy and rapid isolation of target-immobilized beads with a magnet (Joeng et al., 2009). In particular, because the library bound to the target molecule can be easily separated from the unbound one through an external magnet, this method has been commonly used to select a target-specific aptamer. To more effectively select an aptamer, SELEX using a microfluidic or chip system was developed (Ahmad et al., 2011). For example, the DNA aptamer that specifically bound to neurotoxin type B was selected after a single round of selection using a continuous-flow magnetic activated chip-based separation device (Lou et al., 2009). In addition, the screening of C-reactive protein (CRP) specific aptamers has been automatically performed utilizing a microfluidic system and magnetic beads conjugated with CRP (Huang et al., 2010). Microfluidic chip-based SELEX is regarded as a great tool for the selection of aptamers rapidly and automatically.

Immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and the rapid diagnostic test (RDT), have been commonly utilized to diagnose diseases in many clinical fields due to their high sensitivity and specificity. These advantages are very important for diagnostic applications, but they have several limitations. Aptamers have the advantages of being stable at a high temperature, cheap to develop and produce, and useful for various applications. Based on these benefits, the antibodies in ELISA and RDT can be replaced with aptamers (Vivekananda and Kiel, 2006). Moreover, diagnostic sensor systems based on aptamers have been reported using various analytical methods including electrochemistry, colorimetry, fluorometry, and imaging.

For the successful diagnosis of diseases, sensitive, accurate and rapid diagnostic methods are strongly required. To achieve these requirements, electrochemical analysis has been applied, and this method is called an electrochemical biosensor (Zhou et al., 2009). It is an analytical technique that measures the potential, current, and resistance of a sensing system to detect or characterize biomolecules using electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), differential pulse voltammetry (DPV), and so on. These electrochemical biosensor methods have many advantages, such as high sensitivity and selectivity, reproducibility, low cost, rapid response time, and convenient functionalization of electrodes with aptamers. In addition, various nanomaterials, such as gold nanoparticles (AuNPs), are available to increase the sensitivity of the sensing system; and diverse electroactive reporters can be used, according to experimental conditions. Based on these benefits, several electrochemical biosensors for the diagnosis of diseases have been reported. As shown in Figure 4, Lee et al., developed an electrochemical biosensor for engrailed-2 (EN2) based on an aptamer and AuNPs in 2015 (Lee et al., 2015). This aptamer-based sensor (aptasensor) has a low detection limit (5.62 fM) and good selectivity for EN2.

Colorimetry is an optical detection technique that uses color changes. These changes are derived from the presence of biomolecules, and the intensity of the changed color is dependent on the concentration of interest. In this system, AuNPs or polymers have often been utilized as indicators because they cause color changes in specific conditions, such

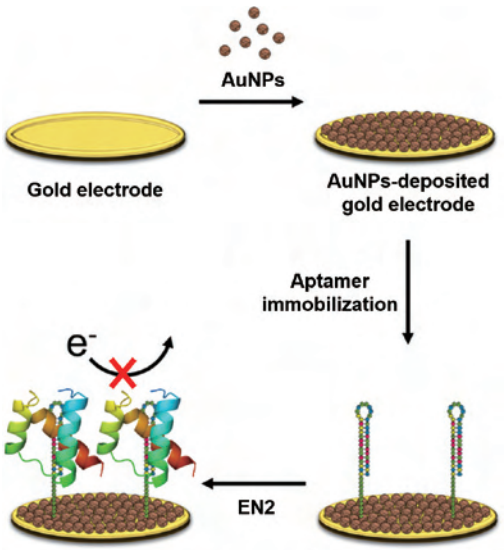


**FIGURE 4 | Detection of the target protein using an aptamer and gold nanoparticle-deposited gold electrode.** First of all, AuNPs are deposited on the gold electrode, and then the target-specific aptamer is immobilized. Target proteins bind to the aptamer, leading to an increase of surface resistance. The image is adapted from (Lee et al., 2015).

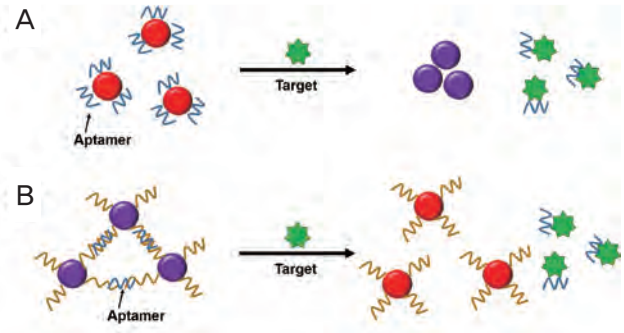


**FIGURE 5 | Schematic illustration of colorimetric aptasensors using gold nanoparticles.** (A) AuNP aggregation by binding between the aptamer and the target. (B) AuNP disaggregation occurs by binding between the aptamer and the target.

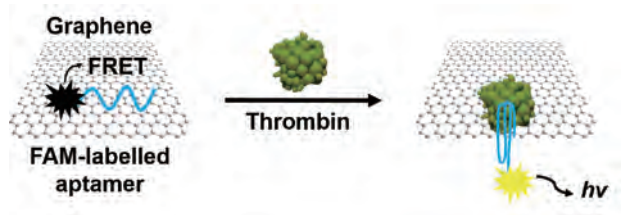


**FIGURE 6 | Schematic illustration of the graphene oxide FRET aptasensor for thrombin.** The image is adapted from (Chang et al., 2010)

as a high salt concentration (Song et al., 2012a). In particular, AuNPs have been widely used in many assays because highly negatively charged aptamers and their complementary strands can protect positively charged AuNPs and are easily immobilized onto the AuNPs' surfaces via the thiol group (Figure 5). In 2010, a colorimetric aptasensor employing AuNPs for oxytetracycline was reported (Kim et al., 2010). In addition, strip-based diagnostic tests are commonly designed using AuNPs. In this type of study, the biomarkers interact with aptamer-AuNP complexes, and then, these complexes are captured by other aptamers on the strip pad. Finally, the aggregated AuNPs on the strip can be observed by the naked eye

Another *in vitro* diagnostic application of aptamers is with fluorescence-based detection techniques. Fluorescence spectroscopy has become a powerful bioanalytical and diagnostic tool in the past 20 years. The most commercially successful application of fluorometry is luminescence immunoassay, followed by the diverse applications of fluorescence-activated cell sorting and other studies on the functions of cells. Other exciting novel areas include fluorescence correlation spectroscopy, which enables the detection of single molecules. As these methods have high sensitivity, new approaches based on aptamers have been reported to combine the advantage of fluorometry with the high selectivity of the aptasensor. In Li's group, a FRET aptasensor for thrombin was developed by combining graphene and a dye-labeled aptamer (Figure 6) (Chang et al., 2010). This FRET aptasensor exhibits high sensitivity and specificity for thrombin due to its high fluorescence quenching efficiency, the electronic properties of graphene, and the quadruplex of the aptamer.

There are many applications of aptamers in diagnostics using various analytical methods, such as surface-enhanced Raman scattering, quartz crystal microbalance, and surface plasmon resonance, in addition to those mentioned above (Song et al., 2012b).

## CONCLUDING REMARKS

In this review, we have focused on nucleic acid-based *in vitro* diagnostics using various techniques, such as amplification, sequencing, hybridization and specific interactions. Furthermore, aptamer-based *in vitro* diagnostics were also introduced. The importance of *in vitro* diagnostics is growing in accordance with the popular demand to improve the quality of life. Through the convergence of diverse systems, such as nanoparticles, microelectromechanical systems, and electroanalytical systems, superior techniques for *in vitro* diagnostics have recently been developed. However, there are some challenges to be overcome, such as the low specificity of biomarkers, the instability of the targets, the occurrence of false-positive errors, and the lack of clear correlations between some biomarkers and prognoses. Furthermore, technical improvements, such as miniaturization of the instrument, development of multi-detection system, and design of fast screening reaction must be accomplished for the fabrication of diagnosis devices. If these drawbacks are resolved, nucleic acid-based *in vitro* diagnostics will become a general tool

for early diagnosis encompassing all types of diseases.


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## AUTHOR INFORMATION

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