Advanced Drug Delivery Reviews 62 (2010) 438–448

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Nanotechnology diagnostics for infectious diseases prevalent in developing countries☆

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ARTICLE INFO

Article history:
Received 8 June 2009
Accepted 14 September 2009
Available online 24 November 2009

Keywords:
Infectious diseases
Diagnostic tests
Nanotechnology
Nanomaterials
Microfluidics
Quantum dots
Metal nanoparticles
Lab on a chip

ABSTRACT

Infectious diseases are prevalent in the developing world and are one of the developing world's major sources of morbidity and mortality. While infectious diseases can initiate in a localized region, they can spread rapidly at any moment due to the ease of traveling from one part of the world to the next. This could lead to a global pandemic. One key to preventing this spread is the development of diagnostics that can quickly identify the infectious agent so that one can properly treat or in some severe cases, quarantine a patient. There have been major advances in diagnostic technologies but infectious disease diagnostics are still based on 50-year technologies that are limited by speed of analysis, need for skilled workers, poor detection threshold and inability to detect multiple strains of infectious agents. Here, we describe advances in nanotechnology and microtechnology diagnostics for infectious diseases. In these diagnostic schemes, the nanomaterials are used as labels or barcodes while microfluidic systems are used to automate the sample preparation and the assays. We describe the current state of the field and the challenges.

Contents

1. Introduction . . . 438
   1.1. Diagnostics for bacterial tuberculosis . . . 440
   1.2. Diagnostics for the human immunodeficiency virus . . . 440
   1.3. Diagnostics for malaria parasites . . . 441
   1.4. Other critical infectious diseases . . . 441
2. Nanotechnology diagnostics for infectious diseases . . . 441
   2.1. Simple nanotechnology-based diagnostics . . . 441
   2.2. Multiplexed detection with nano-diagnostics . . . 443
   2.3. Advanced platforms and read-out systems. . . . 446
3. Conclusions . . . 447
Acknowledgements . . . 447
References . . . 447

1. Introduction

Despite significant advances in antibiotics and diagnostics over the past century, infectious diseases caused by viruses (human immunodeficiency virus (HIV), hepatitis C and dengue fever), parasites (malaria, trypanosomiasis and leishmaniasis) and bacteria (tuberculosis and cholera) are major causes of morbidity and mortality in the developing world [1]. While initial outbreaks of infectious diseases may be

Abbreviations: AIDS, acquired immunodeficiency syndrome; BCA, bio-barcode based amplification; BCG, Bacillus Calmette–Gurin vaccine; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay methods; HIV, human immunodeficiency virus; ICT, immunochromatographic tests; IGRA, interferon gamma release assays; LOC, lab-on-a-chip; PCR, polymerase chain reaction; POTC, point-of-care tests; PPD, purified protein derivative; PSA, prostate specific antigen; QD, quantum dot; SARS, severe acute respiratory syndrome; TB, tuberculosis; TST, tuberculin skin test.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Nanotechnology Solutions for Infectious Diseases in Developing Nations".

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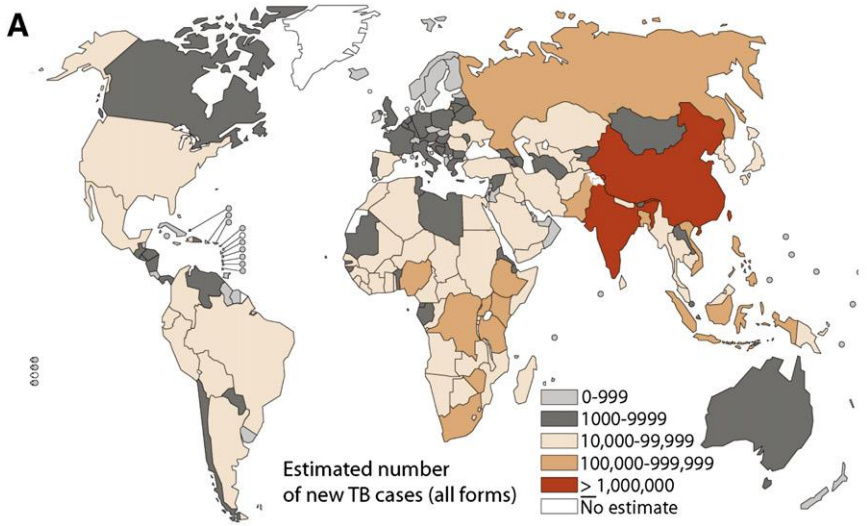
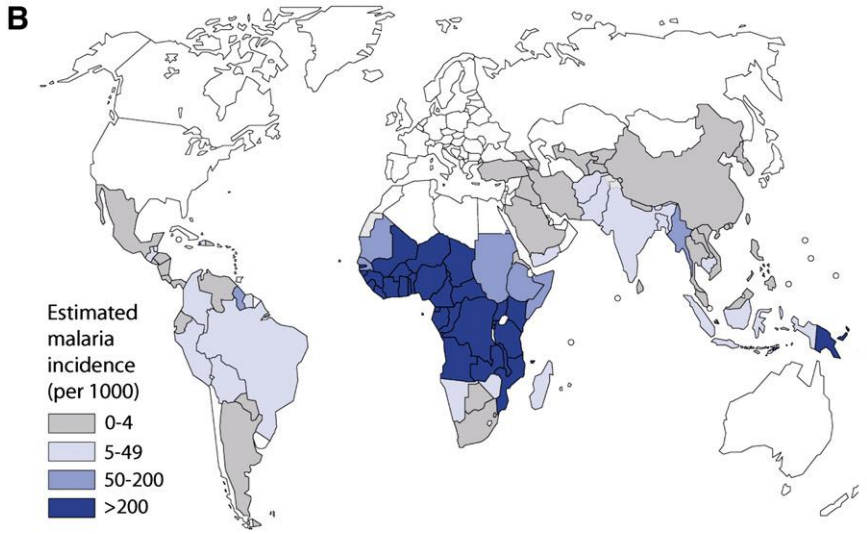
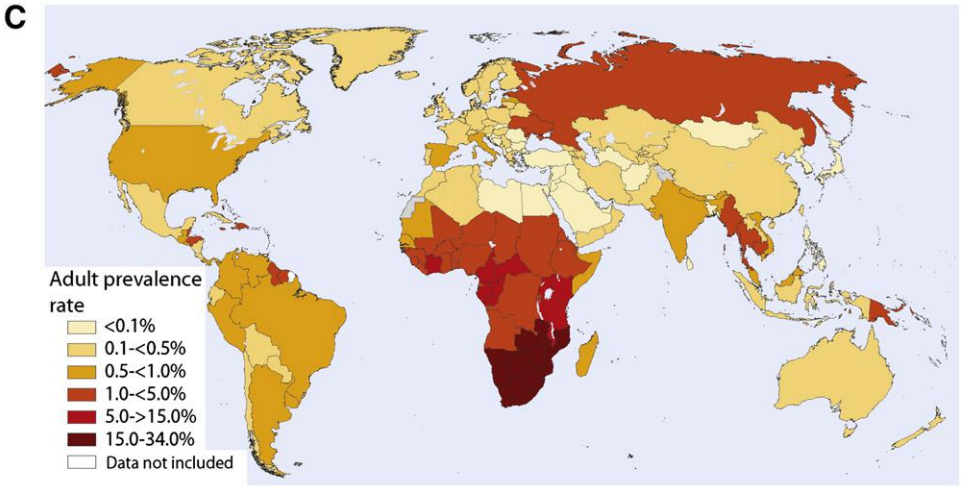
doi:10.1016/j.addr.2009.11.015







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Pathogen	Characteristics	Diagnostic Tests
TB Mycobacterium tuberculosis	• Latent infections difficult to diagnose • Large group of infected individuals • Exacerbated by HIV/AIDS	Microscopy, skin test new Ig λ tests
HIV Human Immuno-deficiency Virus	• Increased risk of other infections • Can be transmitted during childbirth • Multiple strains	ELISA, Western-Blot Rapid Tests
Malaria *Plasmodium*	• Diagnosis often based on symptoms • Emergence of drug-resistant strains	Microscopy, Rapid Tests

Fig. 1. Distribution of HIV, tuberculosis and malaria in the world. These diseases have particularly high incidence rates and prevalence in developing regions such as Africa and South-East Asia. Maps adapted from the World Health Organization. (A) Estimated TB incidence rates, by country, 2007. (B) Estimated incidence of malaria per 1000 population, 2006. (C) A global view of HIV infection (39.5 million people [34.1–47.1] living with HIV in 2006). (D) Key diseases and specific concerns associated with these diseases. Of note, rapid tests refer to the immunochromatographic method or the dipstick test. "Disclaimer: The boundaries and names shown and the designations used on this map do not imply the expression of an opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries."

geographically localized, globalization and international travel can lead to their rapid spread around the world [2,3]. As an example, Fig. 1 shows the spread of HIV, tuberculosis, and malaria around the world [1,4,5].

To combat the spread of infectious diseases, researchers and clinicians require accurate tools for the identification of pathogens so that they can assess the severity of a patient's illness, advise proper treatment, or in some cases, quarantine infectious individuals. Despite the importance of proper diagnosis, infectious disease diagnostics have changed little over the last fifty years. Gold standard techniques in infectious disease diagnostics include microscopy, tissue culture, lateral flow immunoassays (also known as dipsticks or immunochromatographic tests — ICTs), and enzyme-linked immunosorbent assays (ELISA). These techniques are expensive, have limited ability to differentiate between multiple pathogens, are slow, and have a poor detection threshold. Recently, the polymerase chain reaction (PCR) technique has been adapted and utilized for pathogen detection. PCR has a higher detection threshold than previous techniques (e.g., 1 million times more sensitive than lateral flow immunoassays) and can selectively differentiate pathogen strains [6]. However, both PCR and real-time PCR are limited by the same problems as the most commonly used diagnostic techniques (e.g., high equipment and reagent expenses and requirement of skilled technicians) and have other hurdles such as the influence of contamination on the measurement result and analysis times of several hours.

Although these diagnostic technologies are commonly used in the developed world, they are often poorly suited for the developing world, where infectious diseases are a major source of morbidity and mortality and where the availability of clinical and laboratory facilities may be limited. Consequently, the developing world presents novel engineering challenges for diagnostics. The ideal diagnostic device for the developing world would need to be a cost-effective, portable, point-of-source or point-of-care detection system that would be highly sensitive, accurate and could differentiate multiple pathogens. The World Health Organization has highlighted the major design criteria for engineering infectious disease diagnostics for the developing world (see Table 1). In this review article, we focus on the recent developments in nanotechnology-based molecular diagnostics. These emerging technologies could overcome a number of the engineering challenges associated with infectious disease diagnostics in the developing world. Our aim in the first part of the review article is to describe several of the most pressing infectious diseases in the developing world and use them as examples to describe current standards and limitations in infectious disease detection. We then go on to describe the current research efforts in nanotechnology-based molecular diagnostics, focusing on technologies that are relevant to infectious diseases.

1.1. Diagnostics for bacterial tuberculosis

Tuberculosis is caused by *Mycobacterium tuberculosis*. The disease presents mainly in the lungs but can also develop as extrapulmonary tuberculosis in the central nervous or circulatory systems or elsewhere in the body. Tuberculosis infections are spread when an actively infected individual coughs, releasing clusters of bacilli into the air that are inhaled by nearby individuals [8,9]. Once in the lungs, the bacilli are taken up by macrophages, where they may be destroyed. However, bacilli may also remain dormant in lung tissue, in a state known as latent infection. An environmental trigger such as pregnancy, subsequent infection with another pathogen, or impairment of the immune system may reactivate a dormant infection into active disease. Untreated active tuberculosis has a mortality rate of approximately 50% [10–12].

Table 1
The World Health Organization Sexually Transmitted Diseases Diagnostics Initiative, which identifies major desirable parameters of infectious disease diagnostics [7]. (www.who.int/std_diagnostics).

	ASSURED criteria
A	Affordable
S	Sensitive
S	Specific
U	User-friendly (simple to perform in a few steps with minimal training)
R	Robust and rapid (results available in less than 30 min)
E	Equipment-free
D	Deliverable to those who need them

Active tuberculosis is diagnosed by chest radiology and occasionally followed by sputum collection and microbiological culture [8]. Radiological testing has low sensitivity and requires trained staff to operate the imaging system. Tuberculosis cultures are usually more sensitive than chest radiology but the microbiological test relies on bacterial growth and may take several weeks to yield a conclusive result [8,13]. Since tuberculosis is contagious, a level 3 biosafety lab is required for growing the cultures. These requirements render such diagnostic techniques inappropriate for the developing world.

For latent tuberculosis, diagnosis is even more challenging, since typical symptoms (coughing, fever, sputum production, etc.) are not present. The tuberculin skin test (TST) is the current standard test for latent infection. This test measures an individual's immune response to purified protein derivative (PPD) a mixture of more than 200 tuberculosis antigens [14]. Individuals with previous exposure to the pathogen (e.g. latent infection) exhibit an immune response and the formation of an induration (localized inflammation) on the forearm where the antigens were subcutaneously injected. The test is inexpensive and non-invasive but it requires two visits within a specific time period to a clinic. Furthermore, the test often yields false positive results in patients previously vaccinated with the Bacillus Calmette–Gurin (BCG) vaccine, and false negative results in patients co-infected with HIV who have low T-cell counts [9].

Recently, blood-based diagnostic tests with higher specificity have been developed to measure the production of INF-γ by T-cells following exposure to TB antigens [8]. These tests are known as interferon gamma release assays (IGRAs) [14]. A test based on enzyme-linked immunospot assay methods (ELISPOT) is the T SPOT-TB test, and uses RD1 antigens to stimulate T-cells. ELISA-based tests include the Quantiferon test, which measures INF-γ following exposure to the PPD antibody mixture and the Quantiferon Gold (which uses RD1 antigens) [13]. The RD1 antigens (CFP-10 and ESAT-6) are superior to PPD antibodies because they are specific to *M. tuberculosis*. As a result, the immune response to RD1 is also specific to *M. tuberculosis* and there is less interference from previous BCG vaccinations. Nonetheless, patients with compromised immune systems (e.g., patients co-infected with HIV) can give false negative results because these patients tend to have low T-cell counts.

It is clear that there are major bottlenecks in diagnosing tuberculosis and that developments in the diagnosis of TB have been slower than other infectious pathogens. This presents an interesting challenge and opportunity for nanotechnologists. For example, the highly effective Quantiferon Gold assays do not react with PPD antibodies and do not require two clinical visits, but do require some laboratory equipment (such as an incubator). The ability of nanoparticles to potentially detect extremely small quantities of pathogens (explained in the following sections) on a stand-alone platform may be particularly useful in the rapid detection of TB.

1.2. Diagnostics for the human immunodeficiency virus

HIV is a spherical ribonucleic acid (RNA)-based virus that infects immune cells (e.g. dendritic cells, helper T-cells and macrophages). Untreated HIV infections progress to acquired immunodeficiency syndrome (AIDS) which is eventually fatal. The virus impairs and destroys immune cells, significantly weakening a person's immune system and their ability to fight other opportunistic infections. One characteristic of HIV is that the virus has a high mutation rate, which makes treatment and diagnosis challenging. HIV can be transmitted

by contact with blood, semen, vaginal fluids, and breast milk [15,16]. The best method for the containment of HIV is to rapidly detect infections and halt transmission. There is still no cure or vaccine for HIV.

Currently, the gold standard for HIV detection is an enzyme immunoassay (EIA) followed by a Western blot. First generation EIAs detected IgG antibodies, whereas current third generation EIAs detect IgM antibodies produced in response to HIV [17]. These tests require trained laboratory personnel and two clinical visits — one to give a blood sample and a second one to receive results. Rapid point-of-care tests (POCT) are available for HIV antibody detection and have comparable sensitivity (98%–100%) and specificity (86%–100%, with an outlier at 75%) with traditional enzyme immunoassays [17,18]. However, the direct detection of viral RNA is possible 9 days after infection, whereas IgG or IgM anti-HIV antibodies (produced in response to the infection) can be detected by Western blots only 3–6 weeks after infection [17]. RNA tests are currently available but require 4.5 h of processing time by trained staff. The HIV capsid protein p24 is also a target of diagnostics [19] and can be detected approximately 16 days after infection [17].

Rapid tests for HIV RNA or p24 antigen in unprocessed blood would allow for the early identification of infected patients in resource-poor settings. Tests which also rapidly quantify viral load (within the span of one physician visit, e.g. 30 min) would help physicians assess the severity of the infection and guide the selection of the most appropriate treatment regimens, since increased viral load correlates positively with adverse clinical outcomes [20] and is associated with increased transmission of the disease [21]. Viral load is used to assess the success of antiretroviral therapy.

1.3. Diagnostics for malaria parasites

Malaria is caused by a protazoan parasite of the genus *Plasmodium*, of which *P. falciparum* is the most deadly and *P. vivax*, *P. malariae* and *P. ovale* are less severe [22]. Malaria is transmitted by *Anophelene* mosquitoes in tropical regions and is most dangerous in children because of their developing and immature immune systems and in pregnant women due to the recoverable loss of immunity during pregnancy [23]. The disease targets the liver and red blood cells and has a latent phase in the liver which can result in recurrence after many years [24]. A variety of anti-malarial drugs are available but drug resistance has become a major concern due to the indiscriminate dispensing of the most effective anti-malarials in the absence of a proper diagnosis. Currently, many patients in developing nations are treated based on symptoms despite the fact that many other diseases manifest in the same manner.

Microscopy is the gold standard for malarial detection. However, microscopic diagnosis requires highly trained and experienced staff, and several strains may be present in the same host. Morphological differentiation of the four relevant *Plasmodium* species with microscopy is subject to experimental technique and even the influence of anti-malarial drugs on strain appearance [25]. For microscopic analysis, a drop of blood from a finger prick is fixed with methanol on a glass slide and stained with dyes to visualize the parasite by light microscopy [22]. However, microscopy faces a number of challenges. The analysis requires highly trained and experienced staff and detection at low parasitemia is often challenging.

Lateral flow immunochromatographic tests (ICT) have been available for the point-of-care detection of malaria for the last 15 years to address the limitations of microscopy (see Fig. 2 for a description of these tests) [22,26]. These tests are relatively inexpensive and require a small volume of whole blood. Some of these tests are able to differentiate between *P. falciparum* infections and the less dangerous *P. vivax*, *P. malariae* and *P. ovale* infections.

Although POCT for malaria have shown comparable sensitivity and selectivity to gold standard microscopy analysis [27], concerns remain about the risk of false negatives [18,28]. Specifically, there is concern that many POCTs do not provide suggested specificity or sensitivity levels of 90% [29]. While some POCTs do detect multiple strains of malaria [30], ideal tests would also detect co-infections which cause similar symptoms in order to prevent misdiagnosis [31]. This would lead to a reduction in the over-use of anti-malarial drugs which leads to drug resistance [30]. Furthermore, for the detection of low levels of the parasite, PCR is still the most effective technology but cannot be used in resource-poor settings [29].

1.4. Other critical infectious diseases

While tuberculosis, HIV and malaria represent critical pathogenic threats in the developing world today [5] other infectious diseases are also causing considerable morbidity and mortality. These so-called neglected diseases include African trypanosomiasis, Chagas disease, leishmaniasis, dengue fever, schistosomiasis, diphtheria, influenza, measles, cholera and leprosy [32,33]. Rapid POCT for these diseases would improve access to treatment in resource-poor settings and allow clinicians to effectively treat and isolate patients without the need for follow-up visits or expensive laboratory facilities. The ability to correctly identify and differentiate diseases with similar symptoms would reduce pathogen spread and alleviate morbidity and mortality.

2. Nanotechnology diagnostics for infectious diseases

Nanotechnology research and development involves the creation and design of structures with at least one dimension below 100 nm. In this size range, researchers can manipulate the optical, magnetic, and electrical properties of nanostructures by altering their size, shape, or atomic composition [34–36]. Currently, a vast library of nanostructures has been synthesized and documented, with a wide variety of properties and applications [37,38]. Fig. 3 illustrates a variety of nanoparticles with potential biomedical applications.

In addition, nanostructure surfaces can be modified with polymers or other functional groups to improve nanostructure monodispersity or reduce non-specific binding of environmental or biological contaminants (e.g. serum proteins). Surfaces can also be modified with molecules such as antibodies, aptamers, or peptides [39] which allow nanoparticles to target a particular gene, protein, cell or organ *in vivo*. Thus far, researchers have primarily exploited nanostructures in cancer applications where they can act as probes for imaging, as delivery vehicles for cancer drugs [40] and as therapeutics for the removal of tumor cells [41,42]. An important area of focus for current and future nanotechnology research is the development of nanotechnology-based molecular diagnostic platforms. Although only a handful of nanotechnology studies are focused on infectious disease applications, many existing nanotechnology platforms could be adapted for infectious disease diagnostics.

2.1. Simple nanotechnology-based diagnostics

Nanomaterials have been used to construct sensors in three different platforms for simple infectious disease diagnostics: (1) nanoparticle labels in ICT assays, (2) nanoparticle aggregation assays and (3) nanoparticle labels of whole pathogens. The principles of the first platform — ICT assays are described in Fig. 2 [22,26]. These tests are based on the same concepts as ELISA and detect antibodies in blood or other body fluids. Traditional lateral flow assays rely on colorimetric detection that is visible to the naked eye using dyes, latex particles, or small gold nanoparticles as contrast agents. The use of gold nanoparticles in lateral flow tests makes this one of the first applications of nanoparticles in disease detection. Detection limits vary, and depend on pathogen species. For example, several rapid test strips for malaria are capable of sensitively detecting *P. falciparum* malaria at parasitemia levels of $\leq$500 parasites/μL, but for other species of malaria they can only detect levels of $\leq$5000 parasites/μL [43]. Excellent antigen targets are available for *P. falciparum* such as

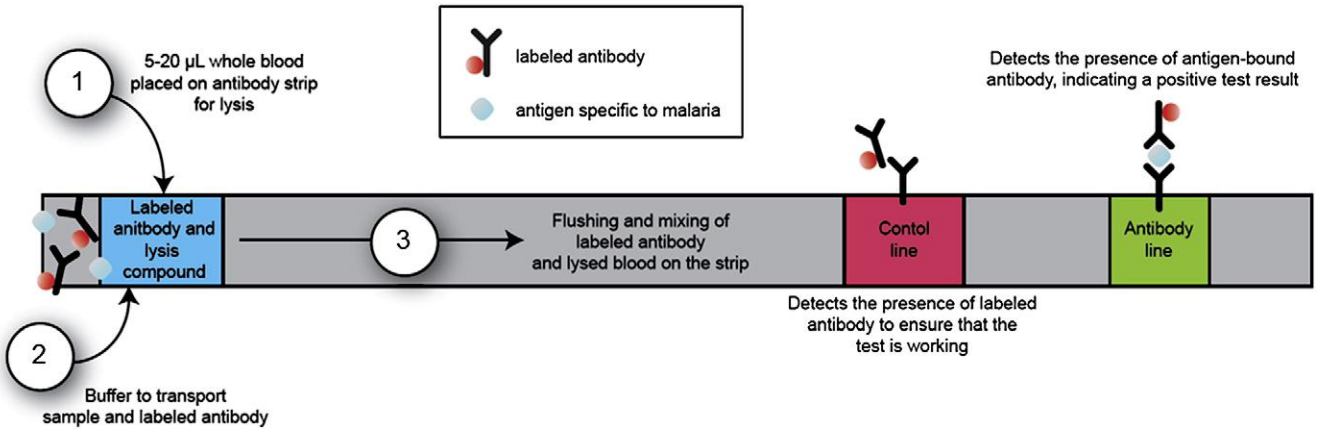


Fig. 2. Immunochromatographic method for rapid test kits. This type of lateral flow diagnostic test is already in use as a rapid test for malaria. Whole blood is placed on an antibody and lysis strip (1), where labeled antibodies contact antigen in the blood if it is present. (2) Buffer is added and transports the labeled antibody to additional antibody lines (4) and (5). Line (4) is a control line, to ensure that the test is working, and (5) is a specific antibody to the target antigen, creating a sandwich assay if the antigen is present. The visual observation of two lines indicates a positive test.
Adapted with permission from Bell et al., Nature Reviews Microbiology 2006, 4: 682–695 [22].

P. falciparum lactate dehydrogenase and histidine-rich protein-2, whereas antigens are not as well-characterized for other malaria species [26]. This highlights the role of the importance of protein and nucleic acid targets in improving the specificity of nanotechnology diagnostics.

Nanoparticle aggregation schemes have been studied for over a decade. Gold and silver nanoparticles have strong optical absorption, which red-shifts following aggregation and provides a convenient optical signal. These detection strategies typically rely on the interaction between nanostructure-bound antibodies and the target molecule [44,45]. The target molecule acts as a bridge between several nanoparticles, and this interaction results in a change in the measurable optical signal of the nanoparticles due to aggregation. One of these strategies, pioneered by Halas, West and co-workers, demonstrated the detection of proteins in saline, serum and whole blood using antibodies conjugated to the surface of gold nanoshells [44]. Upon interaction with the target antigen (rabbit IgG), the anti-rabbit antibody-functionalized nanoshells aggregated to cause broadening of the corresponding nanoshell extinction peak at 720 nm. Changes in optical extinction were measured with a standard UV–vis spectrophotometer. This fast (10 min) assay was able to detect proteins in the range of 88–0.88 ng/mL, which is comparable to ELISA assays but required minimal sample preparation and purification.

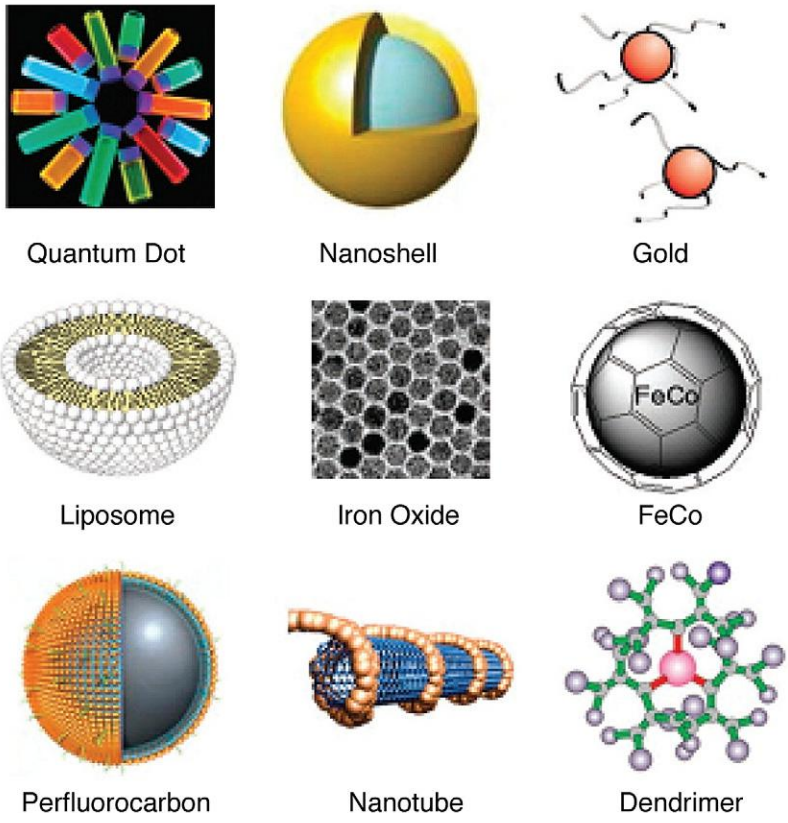


Fig. 3. Nanoparticles currently synthesized for biomedical applications. These include metallic, semiconductor and organic molecule nanomaterials of a variety of shapes, sizes and structures. (Reprinted with permission of the Wiley Publishing Company, Cai et al. Small 2007, 3, 1840–1854 [38].)

For genomic detection, metal nanoparticles can be coated with specific nucleotide sequences, which are complementary to a target genome. In the presence of the target genome the complementary sequences hybridize, linking or aggregating several particles. The interaction of the particles produces a colorimetric change in the solution, which is reversible if the solution is heated to disrupt the nucleotide interactions [46,47]. Mirkin and co-workers successfully demonstrated that 13 nm gold nanoparticles functionalized with a specific oligonucleotide sequence selectively assembled in the presence of a complementary target DNA [48]. The colour of the solution containing nanoparticle aggregates changed from red to blue due to changes in surface plasmons and scattering properties of interacting particles. The nanoparticle-labeled DNA aggregates showed extremely sharp melting profiles when the complementary oligonucleotide strands were heated and separated into single strands. Although the colorimetric assay was capable of distinguishing a single base-pair mismatch in the target sequence, the assay only exhibited a detection threshold in the nM to pM range [49]. This type of colorimetric assay was also utilized in viral detection systems based on supramolecular liposomes. The interaction of specially designed liposomes and virus particles caused a shift in the absorbance spectra of the materials for detection [50].

Direct labeling and detection of pathogenic markers is also a well-characterized method of detecting infectious pathogens. For example, Lieber and co-workers used boron-doped silica nanowires to detect influenza A. In this study, the authors attached an antibody specific to the virus to the nanowires and analyzed the change in nanowire conductivity after antigen–antibody interaction [51]. They achieved measurable changes in conduction at viral concentrations of 100 U per μL. To demonstrate specificity, it was shown that the antibody-coated nanowire did not produce a measurable signal when it was incubated with adenovirus or paramyxovirus. In a study by Tan et al., dye-doped silica nanoparticles were used with an antigen–antibody detection scheme to label *Escherichia coli* in less than 20 min. A particular strain of *E. coli* (OO157:H7) was identified by a specific antibody (mAbs) on the nanoparticle, and a strain without the targeted antigen (ODH5α) was not labeled by the nanoparticles. As few as 400 bacterium per gram of spiked ground beef, could be detected by the assay [52].

While these detection schemes require laboratory equipment such as fluorimeters or spectrophotometers, they do not require highly trained staff such as pathologists or extensive level 3 biohazard facilities. Furthermore, gold nanoparticle aggregation can be detected with the human eye, making direct labeling an appealing modality for the developing world. However, the importance of detecting multiple pathogens and differentiating related or similar infections is also critically important and is discussed in the next section on multiplexed detection.

2.2. Multiplexed detection with nano-diagnostics

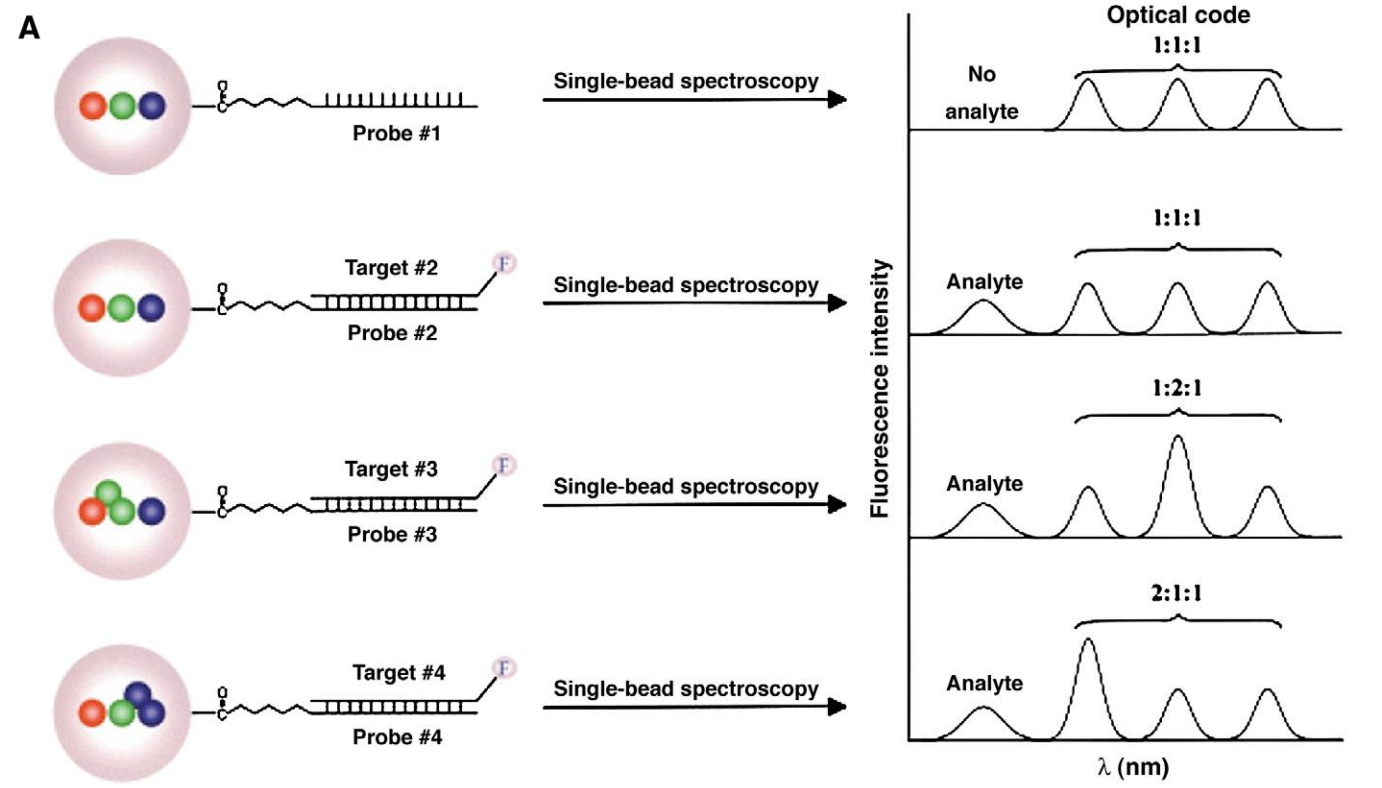
While the simple nanotechnology-based diagnostic platforms have comparable sensitivity to gold standard infectious disease diagnostics, the ability to simultaneously screen for multiple pathogens or markers is limited. Pathogens such as the *Plasmodium* parasites and HIV are present in multiple strains, and the ability to identify the strain could improve treatment efficacy. Currently, a major focus in nanotechnology research is the development of multiplexed assay systems which can detect a multitude of molecules or whole viruses or bacteria simultaneously.

While there are many types of barcodes available [53] we will focus on quantum dot barcodes and the genomic bio-barcode assay (BCA) as an example of the design and use of barcodes in infectious disease diagnostics. The diagnostic principle is similar for all nanotechnology-based barcodes. Semiconductor quantum dots (QDs) are bright, photostable fluorescent markers and have been used in both proteomic and nucleic acid detection schemes. QD barcodes generally consist of polystyrene microspheres containing different ratios of fluorescent quantum dots, with each unique colour label corresponding to a particular antigen or oligonucleotide target. A positive detection event occurs when there is simultaneous detection of the barcode signal and a secondary fluorophore. The "biological entity" to be detected could be gene, protein, or whole pathogen. Fig. 4 illustrates these barcodes.

In a homogenous, solution-based assay, Chan and co-workers developed QD barcodes for the multiplexed and high-throughput detection of biomarkers that code for the blood-borne infectious diseases (hepatitis B (HBV), hepatitis C (HCV) and HIV) [56]. Using two colour (570 nm and 615 nm emitting) ZnS capped CdSe QDs, the researchers created three barcodes that were conjugated with pathogen biomarkers hepatitis B surface antigen (HBsAg) for HBV, HCV non-structural protein 4 (NSP-4) for HCV and HIV glycoprotein 41 (gp41) for HIV. The QD barcodes were incubated in human serum spiked with corresponding antibodies. Sandwich assay complexes were formed during a subsequent incubation with fluorophore-antibody conjugates to provide a fluorescence detection signal peak at ~520 nm. The entire assay can be performed within approximately 1 h. This rapid multiplexed detection scheme exhibited detection thresholds in the range of 10^{-10} M to 10^{-12} M with a sample volume of ~100 μL. The system utilized a chip-based microfluidic device (described in the following section) for readout, and is currently being developed into a portable, point-of-care diagnostic system.

For the identification and quantification of nucleic acids, Nie and co-workers' proof-of-concept study demonstrated the use QD barcodes for multiplexed DNA detection [54]. Uniquely fluorescent QD microbead barcodes were conjugated with capture oligonucleotide strands that could detect complementary strands attached to a fluorophore of a different colour. Fig. 4 shows a direct assay but this assay system could be extended to a sandwich assay format. In related work, Mahoney and co-workers reported QD barcode based, multiplexed single nucleotide polymorphism (SNP) genotyping [57]. They reported a detection threshold of 0.2–1.0 ng of genomic DNA for 10 SNP genotype determinations (total volume 25 μL). This technology could be suited to the rapid identification of different strains of infectious pathogens within a short period of time following infection.

Heterogeneous chip-based assays have also been engineered for multiplexed molecular detection. In one example, Mirkin and co-workers designed a chip-based molecular diagnostic technique using gold and magnetic nanoparticles (see Fig. 5) known as the bio-bar-code-based amplification assay (BCA). In their detection scheme, gold nanoparticles were labeled with two different nucleic acid sequences. The purpose of the single stranded sequence is to capture an oligonucleotide target sequence from the biological sample. The other DNA sequence is the barcode — it is used to identify the target sequence, and is in duplex form. A complementary sequence to the target sequence is on both the gold nanoparticle and on a magnetic nanoparticle and these two structures assemble as a result of nucleic acid hybridization. In a typical assay, a library of gold nanoparticles and magnetic particles is added to a vial and the genetic material of the pathogen (after being processed to isolate and melt the genetic material into single stranded sequences) is added to the solution. The pathogen's genetic material acts as a bridge to join the gold nanoparticles with the magnetic particles. An induced magnetic field separates the bound gold nanoparticles from unbound gold nanoparticles in solution. Afterwards, the sample is heated and the identifying duplex sequence dehybridizes and is captured by oligonucleotide strands on a chip, which are then detected by another gold nanoparticle coated with a matching sequence. Finally, a silver stain is used to amplify the detection threshold. This technique is called the scanometric assay. This technique has a detection threshold of 10^{-21} M, which is equivalent to 10 oligonucleotide copies [58,59]. In 2005, Mirkin and co-workers demonstrated the multiplexed detection of 4 synthetic genetic DNA sequences representing the viruses hepatitis B, variola (small pox),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Single-bead spectroscopy
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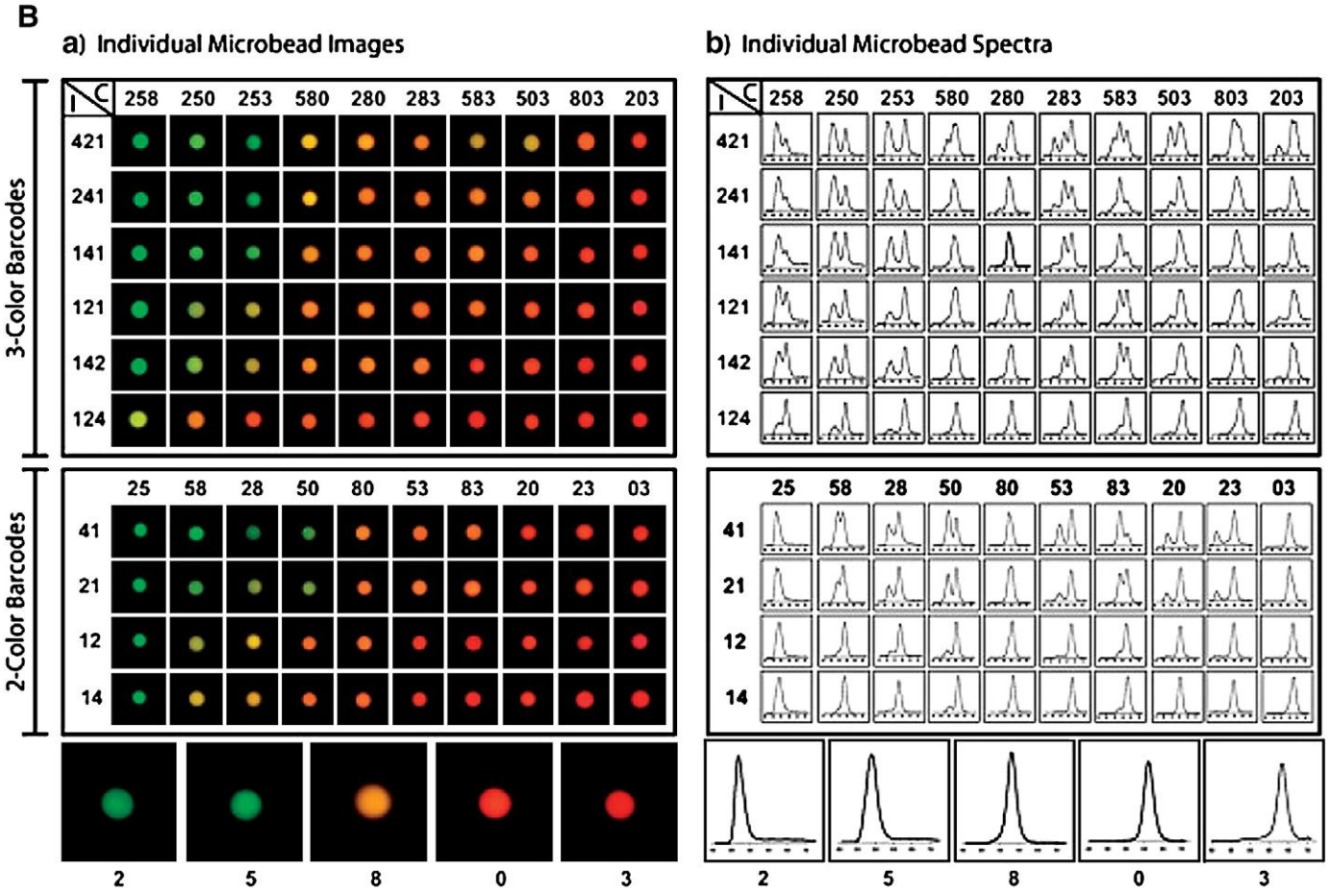
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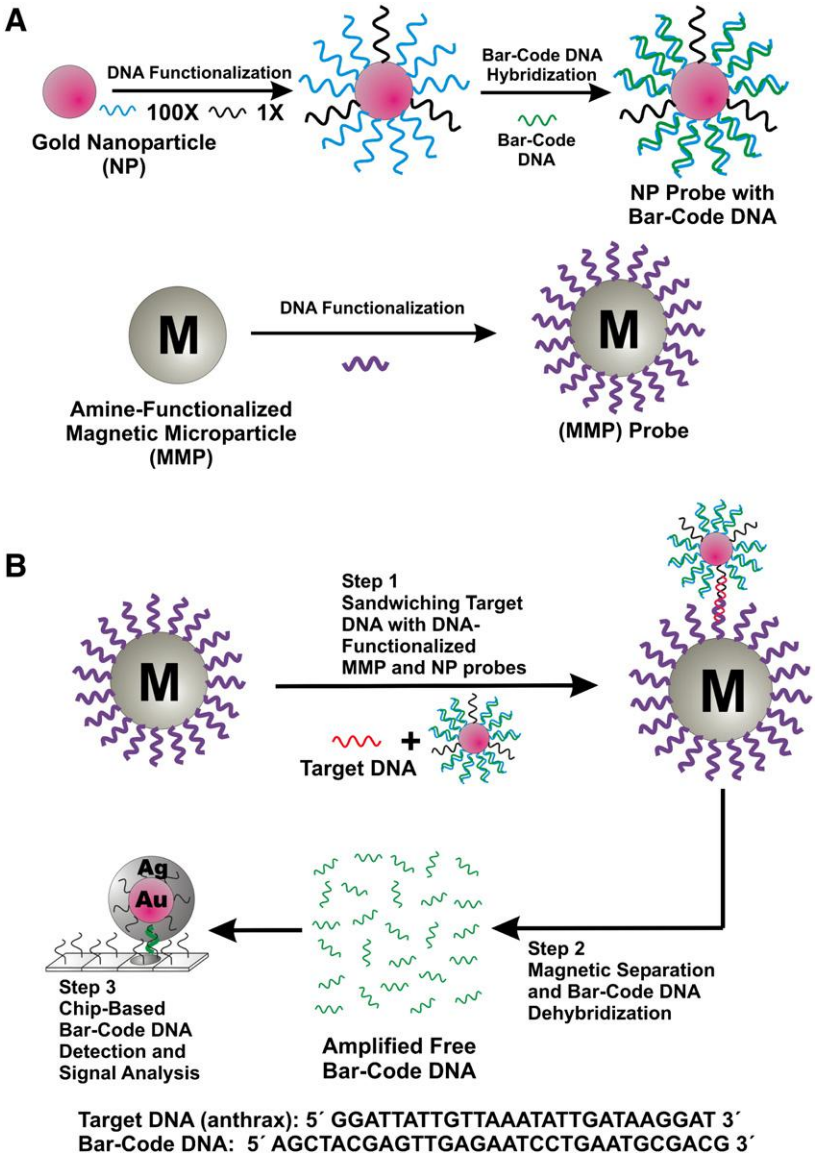

Fig. 5. The principle of a bio-barcode-amplification based assay for DNA detection. (A) Preparation of gold nanoparticle and magnetic microparticle probes. Gold nanoparticles are functionalized with hybridized barcode DNA and capture strands for the target (Anthrax). Magnetic nanoparticles are functionalized with capture strands for the target. (B) Bio-barcode based amplification (BCA) technique coupled with scanometric detection. In brief, the gold nanoparticles and magnetic nanoparticles form a sandwich around the target DNA (since they both have capture strands to it), at which point the sandwich structures can be magnetically separated from the solution and washed. The barcode DNA is then amplified and detected by the scanometric technique. (Reprinted with permission from the American Chemical Society, Nam et al. Journal of the American Chemical Society 2004, 126, 5932–5933 [58].)

ebola and human immunodeficiency virus (HIV) using the BCA method but coupled with scanometric detection at a detection threshold of 500 fM level [60]. However, this method generally requires the use of detectors such as the Verigine system, which measure scattered light from nanoparticle samples. Such systems are expensive, complex bench-top instruments and further development to scale down these devices or the use of simpler systems (such as naked eye readout) is essential for developing world applications.

Although gold nanoparticle-based BCA assays coupled with scanometric detection provide PCR sensitivity levels, the time required for a single assay is still approximately 6 h. Due to the lengthy procedure, this chip-based assay still faces practical challenges before it can be developed for rapid point-of-care diagnostics. In general, these nanoparticle-based multiplexed detection systems have great potential but are still relatively complex and require considerable laboratory equipment. Through commercialization and further research and development these technologies may become feasible for the developing world. Reduced system complexity and the ability to incorporate the detection scheme into a fully closed diagnostic “black box” into which a sample could be placed are important areas of future research. The potential for “labs on a chip” fits these requirements and is discussed in the next section.

Fig. 4. Principle of quantum dot based barcodes. (A) QDs can be used as barcoded probes for detecting multiple targets in a single assay. Barcodes can be generated by varying ratios of QDs colours for each target and thereby creating unique fluorescent intensity signatures. Detection of an analyte occurs when the detection signal is seen at the same time as the representative analyte barcode. (Reprinted with permission of the Nature Publishing Group. Han et al. Nature Biotechnology 2001, 19: 631–635) [54]. (B) Production of 105 quantum dot barcodes for multiplexed detection. This allows for the multiplexed detection of many antigen targets. (Reprinted with permission of the Wiley Publishing Company, Fournier-Bidoz et al. Angewandte Chemie 2008, 47, 5577–5581 [55].)

2.3. Advanced platforms and read-out systems

The use of nanotechnology alone (such as gold nanoparticle assays based on particle aggregation) is promising, but nanotechnology diagnostics also require self-contained platforms for sample processing and read-out analysis. To this end, considerable recent work has shown that nanotechnology experiments can be conducted in microfluidic channels with cross-sectional dimensions of tens to hundreds of microns. In addition, multiple functions can be built into such chips to allow for assay preparation and automation (refer to Fig. 6). These lab-on-a-chip (LOC) systems have many advantages, including reduced consumption of reagents, smaller sample volumes, reduced waste production due to miniaturization, short analysis time, and more importantly, potential for system integration with detection instruments to create portable devices [61]. LOC techniques provide a critical bridging technology [62], and may provide an ideal way of delivering nanotechnology diagnostics. For more information about this technology, please consult some excellent reviews about LOC systems [61,63–65].

Considerable recent work has involved the development of hybrid LOC and nanotechnology devices and nearly every type of nanomaterial reviewed in the previous section can be used in applications involving microfluidic bioassays. QDs have been employed in several microfluidic immunoassay studies either as detection labels for improved detection thresholds [66], or as colour signals for multiplexed detection [67]. One previously-mentioned assay employed quantum dot barcodes in a LOC device as a multiplexing platform to simultaneously detect multiple antigens for HIV, HBV and HCV in human sera [56]. Gold nanoparticles have been extensively used in LOC devices either directly as detection labels [68], or with further catalytic silver enhancement [69,70]. Magnetic nanoparticles have also been used, either to provide a large surface area and flexible magnetic control [71–73], or as the secondary label for detection with magnetoresistive sensors [74]. Surface enhanced Raman spectroscopy (SERS) has been used for a LOC nucleic acid diagnostic assay [75], and the label-free detection method of localized surface plasmon resonance has been used in a microfluidic immunoassay to detect insulin [76].

Despite significant advancements, most LOC assays still rely on bulky and expensive bench-top instruments for signal detection, such as fluorescence microscopy and spectrophotometry. For some studies, portions of the assay were conducted using conventional laboratory methods, such as sample pretreatment for nucleic acid analysis. Although the development of a portable, self-contained LOC device is a challenging task, a few studies of fully or semi-integrated diagnostic devices have been reported. For example, Sia et al. developed a proof-of-concept portable and low-cost immunoassay for resource-poor settings, with straight microfluidic channels [70]. The detection signal was the optical density of a silver film deposited on a polystyrene plate, detected by a custom-built portable battery-powered laser detector (for less than $45 in components). The optical detector demonstrated comparable analytical performance (detection threshold and reproducibility) to a conventional bench-top plate reader for ELISA, and the validity of the LOC assay was demonstrated by measuring the infection status of HIV-1 in human sera. A detection limit of 89 pM could be measured.

Another study developed a diagnostic DNA assay using a single silicon-glass microchamber [77] with the ability to thermally lyse cells, isolate genomic DNA with magnetic particles, amplify DNA by PCR, and electrochemically detect deposited silver. Multiplexed detection was demonstrated with the model analytes *E. coli* and *Bacillus subtilis* pathogens.

The highly sensitive BCA assay mentioned in the previous section has been also developed for microfluidic applications and tested for the detection of prostate specific antigen (PSA) [69]. A compact microfluidic chip permitted the integration of both stages of the assay. Magnetic nanoparticles were used to recognize the antigen target and immobilized with a magnetic field. Gold nanoparticles with barcode DNA and antibodies were then added and also attached to the antigen. Deionized water was used to separate and isolate the double-stranded barcode DNA, which could then be transported to

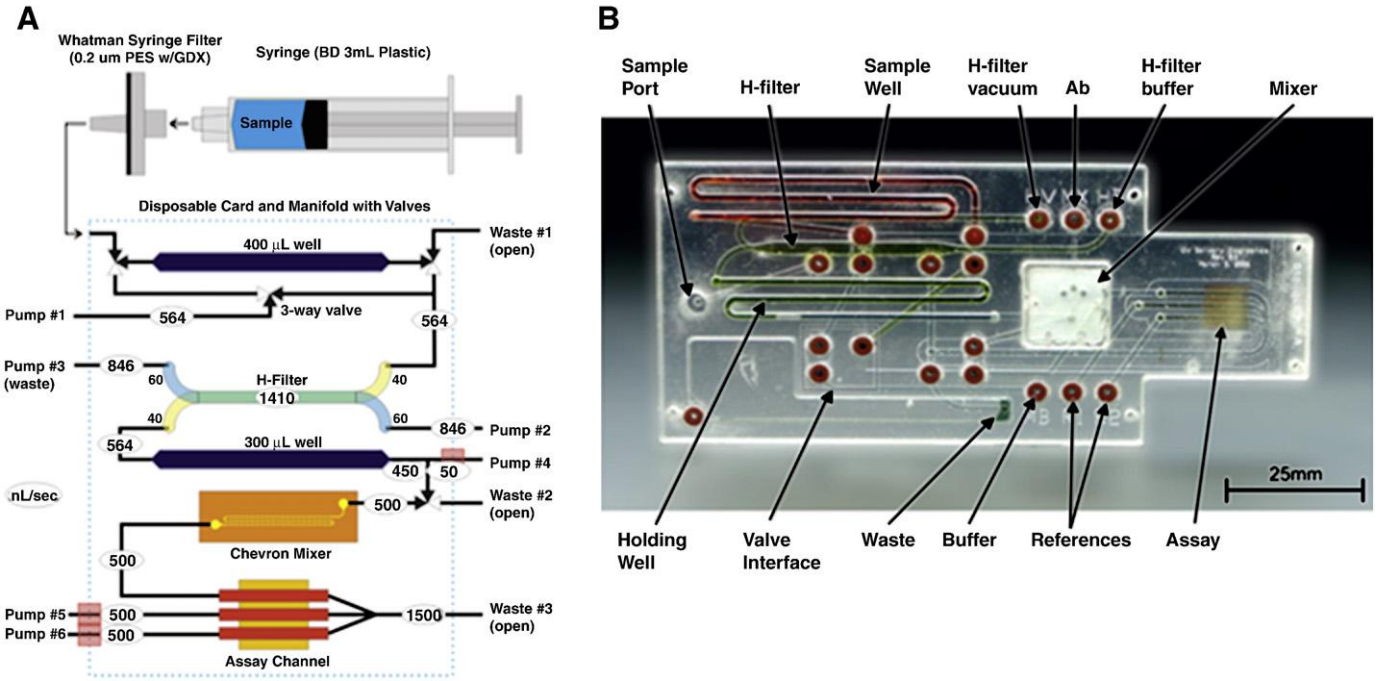

Fig. 6. Images of a diagnostic card, based on lab-on-a-chip technology. (A) Schematic illustrating fluid flow in the card. Miniaturized technology such as the herringbone mixer in the center of the card permits internal sample processing. (B) Actual image of card showing wells, ports for sample addition and reagent wells. Small volumes are required and waste products are sequestered following card use.
(Reprinted with permission from the Nature Publishing Group, Yager et al. Nature, 2006, 442, 412–418 [65]).

the detection section of the chip. Gold nanoparticles with complementary DNA hybridized to the barcode on the detection region, and the addition of a silver stain amplified the optical signal of the gold nanoparticles. The reaction signal (silver stain from catalytic deposition) was detected by using a commercial scanning system, which could be developed into a portable and low-cost platform. The LOC assay was successfully used to detect PSA at a detection limit of 500 aM.

In the integrated LOC studies described above, the signaling reagent is gold nanoparticles and the detection is based on either optical density or electrochemical measurement. These techniques and others, such as magnetic detection or QD fluorescence imaging, have the potential for incorporation into portable LOC devices. Nonetheless, complicated optical systems for fluorescence or spectrophotometry measurement are still very difficult to miniaturize. An increasing number of studies have reported the successful integration of optical nanoparticle detection technologies and LOC chips, some using miniaturized optical devices [78,79], or even incorporating the optoelectronic elements (i.e. photodiodes, waveguides, and lenses) directly into the microfluidic chip [80–83]. Further advances in miniaturization and incorporation are essential for the eventual use of LOC technology in point-of-care diagnostics.

3. Conclusions

Infectious diseases cause millions of deaths a year around the world, especially in developing regions. Appropriate interventions such as targeted treatments are critical, and will only be possible with context-relevant diagnostics, which can economically and effectively identify infected persons. Innovative technologies are needed to make novel advances and permit rapid, multiplexed disease detection, an area in which nanotechnology could play a leading role. Through the use of quantum dots, metallic nanostructures, other nanoparticles, and their integration with lab-on-a-chip technologies, promising diagnostic alternatives are already in development.

The current advances we have discussed include highly sensitive nanoparticle tests, multiplexed homogeneous and heterogeneous systems and lab-on-a-chip platforms. However, there are many challenges which must be addressed before nanotechnology diagnostics are truly ready for use in the developing world. These include the discovery and selection of effective antigen, antibody and nucleotide targets, which are required to improve the specificity of nanotechnology-based detection platforms and permit strain differentiation. Furthermore, universal standards for the assessment of tests and levels of detection must be set so that studies of detection limit can be compared. In addition, continued efforts towards the creation of miniaturized detection platforms are needed. For genomic detection, strategies to simplify the purification and isolate genes of interest are essential.

For the three critical diseases identified, specific aspects of nanotechnology research must be developed. For TB, rapid multiplexed tests are needed to detect simultaneous HIV infections, and the sensitivity of nanotechnology assays must be exploited for the identification of latent infections. For HIV, extremely rapid detection, ideally based on viral RNA, would aid in stemming the spread of the virus, and knowledge of viral load would assist treatment. Finally, for malaria, differentiation of strain and multiplexed detection of other diseases with similar presentation would permit targeted treatment and prevent the emergence of parasites resistant to anti-malarials. Although the advances of nanotechnology have not been fully applied to infectious disease detection in the developing world, nanotechnology can potentially address many of the challenges outlined by the World Health Organization for the delivery of rapid and effective point-of-care diagnostics.

Acknowledgements

The authors thank CIHR, NSERC, and the Canadian Foundation for Innovation for funding. T.S.H. thanks NSERC, CFUW and the K.M. Hunter Foundation for funding.

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