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Research review paper

# Advances in nanomaterials and their applications in point of care (POC) devices for the diagnosis of infectious diseases

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ABSTRACT

Nanotechnology has gained much attention over the last decades, as it offers unique opportunities for the advancement of the next generation of sensing tools. Point-of-care (POC) devices for the selective detection of biomolecules using engineered nanoparticles have become a main research thrust in the diagnostic field. This review presents an overview on how the POC-associated nanotechnology, currently applied for the identification of nucleic acids, proteins and antibodies, might be further exploited for the detection of infectious pathogens: although still premature, future integrations of nanoparticles with biological markers that target specific microorganisms will enable timely therapeutic intervention against life-threatening infectious diseases.



**Contents**



*Abbreviations:* POC, point-of-care; SARS, severe acute respiratory syndrome; HIV, human immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; NAT, nucleic acid test; LOD, limit of detection; RT-PCR, real-time polymerase chain reaction; HBV, hepatitis B virus; HCV, hepatitis C virus; ECM, electrochemical; AuNPs, gold nanoparticles; QDs, quantum dots; MWCNTs, multi-walled carbon nanotubes; HEV, hepatitis E virus; RT-LAMP, reverse transcription loop mediated isothermal purification; NMOF, nano metal-organic framework; SPR, Surface Plasmon Resonance; HbsAg, hepatitis B surface antigens; MNPs, Magnetic NanoParticles; MAP, *Mycobacterium avium* spp. *Paratuberculosis*; BSA, bovine serum albumin.

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## 1. The threat of infectious diseases

According to the World Health Organization (WHO), in 2012 infectious diseases claimed 15 million lives worldwide (World Health, 2013). Among them, Human Immunodeficiency Virus (HIV) and tuberculosis were the leading causes of death at all age groups. In 2011, HIV claimed 1.3 million lives in sub-Saharan Africa alone (Tarantola et al., 1993). The extent of damage exerted by a particular infectious disease could reach well beyond the people directly plagued by the germs. A recent Ebola outbreak caused so much trouble for the healthcare system in West Africa that there were insufficient resources available for measles vaccination programs, thereby further adding to the death toll (Takahashi et al., 2015). An even more recent outbreak is represented by the Zika virus, currently spreading in the Americas and the Pacific region. This has resulted in increased infections during pregnancy and microcephaly, as well as Guillain-Barré syndrome in adults.

The transmission of pathogens is not limited to just humans. A number of transmissible microbes originated from animal vectors (e.g. birds, bats, ticks, etc.) could subsequently switch host to humans. Severe acute respiratory syndrome (SARS) virus, hantavirus, Nipah virus and human immunodeficiency virus (HIV) are just a few of such examples (Morse et al., 2012).

In the past few decades, the spread of once dreaded maladies such as smallpox and poliomyelitis have generally been kept under control, but these rigorous vaccination programs are far from being equally practiced across the globe (Fonkwo, 2008). In developing countries, a lack of proper sanitation, technologies, equipment, and human resources has been hampering efforts to provide timely treatments (Batt, 2007).

## 2. Current diagnostic tools

Identification of microorganisms by observing characteristic features of cultures has been in practice for decades. However, several limitations render this classical technique impractical for on-site diagnosis of infectious diseases, especially in resource-poor regions (Kaittanis et al., 2010).

Being time-consuming is one of the principal flaws of current diagnostic approaches. For preliminary results, each analysis takes 2–3 days. For more definite results, it might take up to 7–10 days. Detection of *Salmonella typhimurium* consumes 3–5 days before yielding results (He et al., 2013), whereas diagnosis of tuberculosis *via* microbiological means may take weeks (Dinnes et al., 2007).

An additional complication derives from the fact that, in order to procure meaningful observations, the initial serum samples must contain pathogen loads above a certain threshold level. This prerequisite might not be met if the patients are in early stages of infection. To worsen the situation, the life cycle of some bacterial strains includes a dormancy state, whereby organisms do not grow significantly in number when cultured. This could culminate in false negative results that critically undermine diagnoses.

Interferon gamma (INF-γ) release assay detects INF-γ produced by T-cells when the patient is exposed to *Mycobacterium tuberculosis* antigen. However, a tuberculosis patient is usually affected by HIV at the same time. Concurrent presence of HIV could readily impair the patient's immune systems. The resulting low T-cell count could mask a clinically relevant quantity of *Mycobacterium tuberculosis*, hence leaving tuberculosis undetected (Diel et al., 2011).

In the case of microbes more diminutive than bacteria (e.g. viruses, with average size of only about one-hundredth that of the average bacterium), an electron microscope is required for detailed visualization of the viral particles (i.e. virions). The growth of viral particles also necessitates a more sophisticated protocol than the one adopted for bacterial cultures (Shinde et al., 2012).

Technological advances have empowered medical professionals with a wide range of diagnostic tools. However, even state-of-the-art techniques are still far from being suitable for application in resource-poor contexts, wherein infectious diseases have proven to be the most widespread.

As of 2007, the gold standard for HIV diagnosis is an enzyme immunoassay which detects IgM antibodies in the patient's serum, followed by Western blot (Branson, 2007). Two popular methods are enzyme-linked immunosorbent assay (ELISA) and nucleic acid test (NAT).

In order to credibly detect a few virions in 100 μl of plasma sample, most commercially available methods require nucleic acid amplification (Calmy et al., 2007, Fiscus et al., 2006, Rouet and Rouzioux, 2007). Fourth-generation ELISA, a combination assay capable of detecting both HIV IgG/IgM and the capsid protein p24, has a limit of detection (LOD) of 4 pg/ml (Speers et al., 2005), thereby removing the need for nucleic acid amplification. The main downside is its high cost.

Amidst the outbreak of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2003, real-time polymerase chain reaction (RT-PCR) (Chan et al., 2004) was widely employed. However, sensitivity of the assay represented the main limitation. In specifics, it would appear below clinically established standards, were the patients infected fewer than six days before the sample extraction date (Vasoo et al., 2009). While a refinement of specimen extraction process does improve the sensitivity level, it leaves the cost issue unaddressed. Another pathogen whose diagnosis utilizes RT-PCR as the standard test is the avian flu H1N1. Commercially available immunochromatography-based strip for the diagnosis of H1N1 (Welch and Ginocchio, 2010) is not as costly, but low sensitivity and specificity limit its clinical utility (Lee-Lewandrowski and Lewandrowski, 2001, Posthuma-Trumpie et al., 2009).

Other than diagnosis, NAT sees extensive use in screening of blood supply for common pathogens such as HIV, Hepatitis B virus (HBV), and Hepatitis C virus (HCV) (Fiscus, Cheng, 2006). It is also employed to monitor patient progress throughout treatment courses. GeneXpert is the first fully integrated NAT system. It could produce test outcomes in 2 h. Despite the relatively shorter assay time, the problems of cost and energy consumption remain (Meyer-Rath et al., 2012).

## 3. Point-of-care (POC) tests

### 3.1. Background information

According to the College of American Pathologists, POC testing could be considered as on-site diagnostic tests carried out using mobile devices readily accessible to the patients and the in-charge physicians (Lamb et al., 1995). Another more concise definition is 'testing done in the proximity of patient care' (Kiechle et al., 1990). The portable devices employed can be either hand-held or transported on a cart (Urdea et al., 2006). The acronym "ASSURED" was coined by WHO to denote the fundamental criteria of POC testing: affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end user (Sista et al., 2008).

As mentioned above, there is an increasing demand for diagnosis of infectious diseases in resource-poor regions. A paucity of laboratory technicians with necessary know-hows is, among others, a major