NANODIAGNOSTICS: A NEW FRONTIER FOR VETERINARY AND MEDICAL SCIENCES

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ABSTRACT

Infectious diseases are one of the greatest threats to animal and human population living in the developing world. These diseases have capacity to instigate in a small area and then open out very fast to the rest of the world and causing a heavy pandemic situation, for example; avian influenza pandemic. Such diseases infect large masses of population and may lead to loss of lives and also incur huge economic losses. Therefore, the best way to control these diseases is by diagnosing it at a very primary level and taking necessary precautionary measures so as to avoid the spread. Since last few years, the diagnostic approach has changed from tedious molecular biological techniques, to easy and rapid diagnostic techniques. Nanotechnology has extended the molecular diagnostics limit to nanoscale. These developed techniques do not require sophisticated laboratories and expert personnel, and hence are a cheap diagnostic approach. These assays can also be performed at the field level where the patient is present and get the results there itself. Hence, they are also called as pen side test or lab on chip diagnostic assays. The biological tests using nanotechnology become quicker, more flexible and more sensitive. These techniques have greatly influenced the diagnostic approach in the veterinary as well as medical field. Especially in the developing countries such as India, where the laboratory services are not

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available at the village level, these techniques have facilitated the disease diagnosis the most. Nanotechnology also applies the tools and processes for nanofabrication which is used to build devices for studying biosystems. Molecular diagnostics incorporated with nanobiotechnology has improved clinical diagnosis and opened a new area for development of personalized medicine. Nanotechnology has also played a crucial role in designing of diagnostic assays for medical and veterinary use. The nano materials have many versatile optical properties, piezo-electric properties, thermal properties, electro-chemical properties, enzyme mimicking properties etc. By exploiting these properties, the workers have designed different approaches for diagnosis. In this review, different nano-diagnostic approaches for detection of pathogen have been stated.

1 Introduction

Bacteria, viruses and other microorganisms are omnipresent creatures which are responsible for causing disease in the humans and livestock. These organisms may affect multiple host species including humans. Therefore, they are of zoonotic importance and important in the public health concern. Some infectious agents can also be used as a part of biological warfare agent (MacKenzie, 2015). Hence, the correct diagnosis of the infectious agent gets primary importance, especially in case of livestock, because they are directly or indirectly linked to the humans through food webs. Several reasons can be attributed towards the diagnosis such as sub-clinical infections, persistently infected animals (PI), carrier or reservoir hosts, organisms transmitted through insect vectors or intermediate hosts (Rivera-Benitez et al., 2016; Navarro et al., 2016; Weber et al., 2016).

Therefore, if the infection can be detected at the very primary level before maximum population is affected, proper control measures can be planned and huge economic losses can be prevented (Cascio et al., 2011; Stephen et al., 2015). Biosensors are commonly used in medical and veterinary diagnostics because of their higher sensitivity, simplicity in operation, ability to perform multiplex analysis, etc. (Patel et al., 2016). Since last two decades tremendous research in the field of diagnostic science has resulted in the development of numerous tools for detection of pathological agents and various diseases they cause in the humans and the animals. These new techniques have so many advantages over the previous techniques (Wei & Erkang, 2013). They are very handy, can be performed and interpreted by a layman, do not require sophisticated laboratories, very quick results with good specificity and sensitivity at a very cheap and affordable rate.

Besides, there is no need of transportation of samples to the labs, as the test can be performed at the point where the animal is standing, thus reducing sample upset (Baptista, 2014; Alharbi & Al-Sheikh, 2014). Meanwhile, there is risk of spread on infectious disease, severe diseases conditions and even death due to absence of appropriate control measures (Dahlhausen, 2010). Apart from delayed diagnosis, other disadvantages such as possibilities of variations induced by transportation of samples, processing and testing conditions and even lack of uniform diagnostic platforms may further

complicate the result and results generated may be doubtful. Now there are different strategies designed for the diagnosis of disease either by detection of Ag or Ab, for which different types of biosensors are designed. In a Biosensor the physiological interaction between the ligand and the bio-recognition element is converted by transducer, into measurable electric signal which can be further enhanced by a computer aided readout system for the user or sometimes can be read by naked eye only (Arora et al., 2010). Generally for the diagnosis of the disease, Ab based biosensors are preferred (Conroy et al., 2009). Mostly, the sensors are designed to diagnose the disease of veterinary importance as well as having zoonotic importance and vice versa (Stringer et al., 2008; Tran et al., 2012). Some have developed the sensors for surrogate human viruses so as to avoid the direct contact with the human viruses (Connelly et al., 2012). Therefore we need other techniques which can diagnose the disease at the point where the patient is present. Such techniques are the requirement for the developing countries like India.

Nanotechnology is an emerging field which has contributed the most for the development of the biosensor technological approach (Syed, 2014). A biosensor is a compact analytical device which employs a ligand-specific bio-recognition element, such as an antibody, enzyme, receptor, nucleic acid, aptamers, peptide/protein, cells, tissue or whole organisms. These elements are immobilized on a sensor surface which is integrated with a signal conversion unit or transducer (Ayyar & Arora, 2013). Nanotechnology employs use of nanomaterials which exhibit physiochemical properties such as electrochemical (Rathee et al., 2016), chemical luminescence (Roda et al., 2016), optical (Tereshchenko et al., 2016), which are completely different than the actual material (Krejčová et al., 2015).

These properties are generally exploited in designing of biosensors. These days even smartphone integrated biosensors have developed (Diming & Qingjun, 2016; Cevenini et al., 2016; Roda et al., 2016). There are many reports on nanoparticles having properties mimicking the properties of certain enzymes, thus these particles can be used in designing immunoassays. In this review, the Nano-diagnostic biosensors for the detection of pathogens which are human and veterinary importance are discussed.

Figure 1 Types of Nano-diagnostic Biosensors

There are various approaches being used for the development of nano-diagnostic assays. The nano diagnostic can be classified into two categories, in-vitro and in-vivo. In-vivo is the diagnostic imaging techniques in case of live animals. On the other hand, the in-vitro techniques include, different antibody based immune assays and different nucleic acid based hybridization assays coupled to the nanoparticles (Figure 1). Several types of biosensor technologies have been used for detection of biomolecules.

2 Immuno assays

These are the label free assays which can detect the substrate without labeling the biomolecules with any enzyme. The Ag-Ab reaction is detected by exploiting diverse properties of nanoparticles. Previously, immuno sensors exploited the very specific binding affinity of antibodies for a specific compound or antigen.

The binding of antigen to antibody follows the lock and key hypothesis of interaction. The antigen-antibody binding usually result in generation of a detectable signals from secondary molecules such as enzymes, fluorescent molecules or radioisotopes tagged with either antigen or antibody (Marazuela & Moreno, 2002).

But due to advancements in nanotechnology, the need of labelling the biomolecule with enzyme or radioisotope is not required when Nano-particles are used (Tianshu et al., 2015). Several types of antibody/antigen interaction detection systems are available which are currently used for detecting diseases, (Table 1, Figure 2). IgG antibody based detection systems have been developed for diagnosis of autism (Gogolinska & Nowak, 2013). For antigen/antibody based detection several types of silver and gold nanoparticles are used. Similarly, silver nanoparticles have been used for diagnosis of H1N1 virus (Yanxia et al., 2014) and gold nanoparticles have been used for diagnosis of Salmonella (Giyoun et al., 2015), Human T lymphotropic virus and Hepatitis B Virus (Randolph et al., 2016) etc (Table 2).

Figure 2 Different approaches for designing antigen/antibody based nano-diagnostic tools.

Table 1 Lateral Flow assay for detection of various biological agents.

Agent	Nanoparticle	Detection Limit	Reference
HIV-1	GNP	0.24pg/ml	Xiuli et al., 2016
HIV MYO	GNP	1.56ng/ml	Ruihua et al., 2016
Mycoplasma pneumonea	AF-647	0.3830	Liming et al., 2016
TB	GNP	100pg/ml	Corstjens et al., 2016
Prostate specific Ag	Photon up-converting NPs	41ng/liter	Juntunen et al., 2016
Hepatitis C	GNPs	-	Hwan et al., 2015
Enterobacteriaceae	GNP	-	Jyoti et al., 2015
Mycotoxin	MNP		Xie et al., 2015

Table 2 Antigen/antibody interaction based system for detection of different pathogens.

Organism	Nano Particle	Type of detection	Ag/Antibody	Reference
Adeno virus	Triangular AuNPs	Raman Scattering	Polyclonal	Chia et al., 2011
H1N1	Silver NPs	Fluorescence OPDA	Monoclonal	Yanxia et al., 2014
Encephalomyocarditis virus	Triangular AuNPs	Raman Scattering	Polyclonal	Chia et al., 2011
Salmonella	AuNPs	Microfluidic	Polyclonal	Giyounget al., 2015
Duck Hepatitis virus	Silicon wafers	Ellipsometry Imaging	Polyclonal	Cheng et al., 2011
HIV	Fe-Au shell	Amperometric	Glycoprotein 160	Ning et al., 2009
Salmonella pullorum	Blue Silica & MNPs	Sandwich assay	Polyclonal	Qian et al., 2016
Salmenella	Quantum dots	Magnetic sensor	Polyclonal	Giyounget al., 2015
Human T lymphotropic virus	GNPs	Immunoaffinity assay	Monoclonal	Randolph et al., 2016
Hepatitis B Virus	GNPs	Immunoaffinity assay	Monoclonal	Randolph et al., 2016
Orchid Virus	Gold Nano rods	SPR	Label free	Lin et al., 2014
General Virus	GNP Chip	Fluorescence	Fluorescence Microscopy	Yen et al., 2016
H1N1, H5N1, H7N9	ZnO Nano rods	PDMS	Electrochemical	Ji-Hoon et al., 2016
H1N1	GNPs	Micro fluidic system	Aptamers	Tseng et al., 2016

2.1 Optical Biosensor

The optical properties of nano-particles are exploited in an optical biosensor (Radhika et al., 2012). The Optical biosensors utilize several sensor techniques such as resonant mirrors, surface plasmon resonance and waveguides can be widely used for analysis of biomolecular interactions without using any molecular tag. Advances in instrumentation and experimental design have led to the increasing application of optical biosensors in many areas of diagnosis (Matthew, 2002). This means that when the conjugated nanoparticles bind to the

specific molecules, they change their refractive index (Xudong et al., 2008) and therefore, change their color which is directly proportional to the number of interacting molecules or mass of the interacting molecules at that given instant. The techniques such as immune dot-blot assay, lateral flow assay work on the same principle. Several types of biosensors have been designed on optical detection principles (Figure 3), such as Surface plasmon resonance based biosensors; interferometer-based biosensors and optical waveguide based biosensors etc (Jeremy, 1997; Baird & Myszk DG, 2001).

Figure 3 Basic principle of biosensors

Figure 4 Surface plasmon resonance based principle.

2.1.1: Surface plasmon resonance (SPR) biosensor

It was first demonstrated for biosensing in 1983 by (Liedberg et al., 1983). Nanoparticles display unique physical properties due to their nano-size. Metallic nanoparticles have intense absorbance and scattering properties due to Surface Plasmon Resonance (SPR). When an oscillating electric field interacts with the free conductive band of electrons at the surface of the AuNP, collective dipolar oscillation of the electrons occurs. This is called Surface Plasmon (Radwan & Azzazy, 2009). SPR has been extensively explored and has gradually become a very powerful label-free tool to detect the pathogens (Pattnaik, 2005; Homola, 2003). In SPR, a surface plasmon wave (SPW) which is a charge density oscillation occurs at the interface of two media with dielectric constants of opposite signs, such as a metal (gold or silver) and a dielectric (Figure 4).

This technique has been successfully used for the detection of viruses and bacteria (Boltovets et al., 2004). Gold nanoparticles embedded PVA matrix is used as sensing material (Rithesh et al., 2016). Detection can be performed by visual colour change observations, photometry or resonance light scattering by interacting molecules on surface of nanoparticles deciphered by changing refractive index. This has a very wide range of applications in the areas of environmental, pharmaceutical and biological analysis and clinical diagnosis (Yanlin et al., 2016). Gurpreet et al. (2016) has reported the use of this type of biosensors in the detection of *Niesseria meningitidis*.

SPR sensors can visualize living cell interactions which can be used for malignant cell detection in cellular diagnostic systems (Yanase et al., 2014). SPR based rapid immunoglobulin M (IgM) diagnostic test has been successfully used for detection of dengue from human serum in only 10 minutes with 100% specificity and 83-93% sensitivity (Jahanshahi et al., 2014). The SPR biosensor based assay was also used for simultaneous

detection of multiple TB antibodies in patient serum with high sensitivity and specificity in real-time (Hsieh et al., 2012).

2.1.2 Interferometer-based biosensors

Optical interferometers have already used in detection of surface bound bio-reactants such as bacteria, spores, toxins, viruses, and proteins (Schneider et al., 2000; Schmitt et al., 2007). These devices are based on evanescent field sensing. Light is confined within the core of the waveguide, and extends into the surrounding media so that its field can interact with the environment. Therefore, a biomolecular interaction takes place between a receptor molecule, previously deposited on the waveguide surface, and its complementary analyte produces a change in the refractive index at the sensor surface that induces a variation in the optical properties of the guided light via the evanescent field. Interferometric assays have an advantage in detection of intact bacterial or viral particles. Influenza virus has been detected in oral-nasal secretion of patients at concentrations of a few ng/mL through this technique. Recent study shows that microorganism growth can also be detected using hollow-core photonic fiber based Fabry-Perot interferometer (Xiaohui et al., 2016). A label-free DNA biosensor based on microfiber-assisted Mach-Zehnder interferometer for in-situ real-time DNA hybridization kinetics detection has been experimentally demonstrated by (Binbin et al., 2016). While Mach-Zehnder interferometer point-of-care system for rapid multiplexed detection of microRNAs in human urine specimens is done by (Qing et al., 2015). Sandwich assay for detection of Streptavidin was demonstrated by (Wenjie et al., 2016) with detection limit of 0.02 nM. The Interferometric biosensor was used for detection of Aflatoxin M1. The test result was highly reproducible and reusable (Chalyan et al., 2016). A fiber-optic interferometer based optic biosensor operating at 1550 nm was evaluated for quantification of gelatin (protein) in water (Yadav et al., 2014).

Table 3 Enzymatic interactions based detection of different agents associated with health concern.

Compound	Nanoparticle	Type of sensor	Detection molecule	References
Norepinephrine	FeMoO ₄ rods	Cyclic voltammetry	Without modification	Kunda et al., 2016
IFN Gamma	AuNP	ITO	HPR-Ab	Yaru et al., 2016
Protein estimation	MNPs	Colorimetric	Punctates	Gero et al., 2016
IL-3	AuNP	iPCR	Polyclonal Ab	Lucie et al., 2011
Stem cell factor SCF	GNP	iPCR	Polyclonal Ab	Lucie et al., 2011
Nano Mass	Graphene films	Ultrasound frequency shift	Piezoelectric crystal	Li & Wang, 2016

2.1.3 Optical waveguide based biosensors

Optical waveguides based biosensor utilize fluorescence resonance energy transfer (FRET) triggered by the binding event between multivalent protein and dye-tagged receptors (Song et al., 2000). It is successfully adapted to the detection of biomarkers for complex biological material. The spatial filtering of wave-based detection is a distinct advantage as it ensures that the bulk biological material is not irradiated. This arrangement effectively minimizes background fluorescence and eliminates the need for extensive sample preparation when analyzing complex samples. Mukundan et al. (2009) have successfully used this approach to detect extremely low concentrations of disease biomarkers in patient samples. Optical wave guide biosensors are used for the detection of RNA in the samples (Carrascosa et al., 2016).

3 Enzymatic interactions based nanodiagnosics

Enzymes are very popular bioreceptors due to their specific binding capabilities and catalytic activity. Enzymatic interaction is used for specific analyte recognition (Pohanka, 2013). The enzyme based biosensors provide specific advantages such as ability to catalyze several reactions, can detect many analytes such as substrates, products, modulators

and inhibitors. Moreover, enzymes are not consumed in reactions. Therefore, biosensor can be used continuously without loss of activity. Enzymatic interactions methods can detect much lower limit of analytes (Patel et al., 2016). However, the sensor lifetime depends on enzymatic stability (Lucie et al., 2011). There are several types of enzymatic interactions detection systems are available which are currently used for detection of agent associated with health concern (Table 3).

Several biological molecules such as IL-3 (Lucie et al., 2011), IFN Gamma (Yaru et al., 2016), total protein (Gero et al., 2016) etc., in disease conditions have been estimated using enzymatic interaction based biosensor. Recently, there has been little advancement in these types of biosensors like, the accumulation of insulin causes type 2 diabetes. To detect this condition a biosensor called Nano-cage-mediated refolding of insulin by PEG-PE micelle has been developed (Xiaocui et al., 2016). Cholin a breast cancer marker, detected from serum by nano interface technology (Thiagarajan et al., 2016). Similarly, blood glucose level is monitored by a noninvasive saliva biosensor (Wenjun et al., 2015). Aptamer based GnRH biosensor in equine urine has been demonstrated by (Richards et al., 2016).

Figure 4 Approaches for making Nucleic acid based diagnostics

Table 4 Nucleic acid interactions based nano-diagnosis detection of different agents associated with disease.

Organism	Nanoparticle	Sensor type	Nucleic acid	Reference
Arabis Mosaic Virus	SMP	Magnetic	RNA	Ning et al., 2014
Lily Symptomless Virus	SMP	Magnetic	RNA	Ning et al., 2014
HSV	SMP	Magnetic	RNA	Ning et al., 2014
GYSVD	SMP	Magnetic	RNA	Ning et al., 2014
HBV	AuNP	Barcode amplification	DNA oligos	Wang et al., 2010
HBV	MNPs	Hybridization	DNA oligo	Wang et al., 2010
Dengu	3D Graphene	Impedimetric sensor	RNA	Seon et al., 2016
Canine adeno	GNPs	Microarray chip	DNA	Yadav et al., 2015
Salmonella	GNPs	LFICA	16s rRAN	Cheng et al., 2013
HBV	Cu Nano cluster	Colorimetry	DNA	Xiaoxia et al., 2016
Influenza virus	CdZnTeS Quantum dots	Fluorescence	Molecular beacons	Oluwasesan et al., 2016
White spot syndrome virus	GNPs	LAMP	DNA oligo	Yortytot et al., 2013
Porcine epidemic diarrhea	GNPs	Nano RT-PCR	RNA	Wanzhe et al., 2015
Influenza	Sugar chain GNP	RT qPCR	SYBRgreen	Yasuo et al., 2015
HCV	GNPs	Hybridization	5'UTR DNA	Sherif et al., 2010

4 Nucleic acid interactions based nanodiagnostics

The nucleic acid based Biosensors are known as genosensors. The analyte recognition is based on principle of nucleotide base pair complementarity, such as A: T and C: G in DNA. Complementary (probe) sequences are synthesized from target nucleic acid sequence, labeled with suitable dye and immobilized on bio sensor chip. Thus, probe will hybridize with target gene followed by generation of optical signals (Marazuela and Moreno, 2002). There are several types of Nucleic acid (DNA/RNA) interaction detection systems available which are used for detection of several viruses or other disease associated agents (Table 4; Figure 4).

The DNA genosensors can be combined with PCR amplification for detection of several microorganisms. The DNA genosensors based assays lead to direct detection of hybridization process using electrochemical redox mediators, enzyme amplification or nanoparticles labeled ingredients (Pedrero et al., 2011). Nucleic acid based biosensors have also used for screening of allergens in food materials because of high stability of DNA in comparison to proteins even after processing of food (Mafra et al., 2008). The assay is based on selection of DNA target sequences coding allergenic proteins.

Such techniques are also used for animal meat identification. Bovine and sheep meat samples were detected by targeting highly repetitive satellites DNA (~250 bp and 430 bp, respectively) (Mascini et al., 2005). A more reliable and faster genosensors based technique has been developed for chicken, bovine and swine meat identification. This method uses a combination of isothermal amplification of DNA along with electrochemical detection of DNA on disposable carbon based electrochemical printed chips (Ahmed et al., 2010). Genosensors are also used for monitoring of genetically modified organisms (GMO) having specific genes (transgene) introduced into their DNA using genetic engineering to

improve crop production (by insect or herbicide resistance) or to enhance nutritional properties. Target gene selections for such genosensors are relatively easy because the transgenic inserts sequences are completely known and available in open databases. Several genosensors have been developed for detection of transgene from GMOs (Yang et al., 2007a; Yang et al., 2007b; Yang et al., 2008; Feng et al., 2008; Jiang et al., 2008; Ma et al., 2008; Zhang et al., 2008; Yang et al., 2009; Zhou et al., 2009; Bonanni et al., 2009; Jiang et al., 2011; Yang et al., 2012; Arugula et al., 2014; Manzanares-Palenzuela et al. 2015).

5 DNA based nanotechnology

DNA nanotechnology utilizes newly designed artificial nucleic acid structures for analytical purposes. In such assays, nucleic acids are used as non-biological engineering materials rather than as carrier of genetic information. Some researchers have designed static structures with DNA, such as DNA computers and molecular machines (Seeman & Nadrian, 2004). There are different DNA based technology such as Microarray, Rolling circle amplification, Threshold mediated strand displacement (TMSD) and L shaped DNA probes in which nanoparticle were used to facilitate the process (Shi et al., 2014; Ravan, 2016; Elham et al., 2016) (Table 5). The nano-biotechnology system may be used for creation of a DNA robot which can recognize infected cells and induce apoptosis to kill such cells (Douglas et al., 2012). The DNA robot was an elegant model system which has shown great potential for uses as a smart drug. The DNA nanotechnology science has also been used as carriers for Doxorubicin (anticancer drug) (Jiang et al., 2012; Zhao et al., 2012). This showed increased potency of Doxorubicin as compared to normal medication. Thus, DNA nanotechnology has shown breathtaking pace in recent years. It leads to control of structure and function at molecular level with unparallel efficiency (Tørring & Gothelf, 2013).

Table 5 Nanoparticles facilitated nucleic acid based technologies.

Technique	Nanoparticle	Sensor type	Nucleic acid	Reference
Micro Array	GNPs	Pixel sensors	DNA	Wang et al., 2010
Rolling circle amplification	GNPs	SPR	DNA probes	Shi et al., 2014
Threshold mediated strand displacement	GNPs	TMSD	RNA	Ravan, 2016
L shaped DNA probes	GNPs	Hybridization	DNA	Elham et al., 2016
RNA quantification	GNPs	Colorimetry	PNA peptide nucleic acid	Joshi et al., 2013

Nano-Immuno-PCR

Nano-Immuno-PCR has additional sensitivity than other conventional methods because it utilizes combined effect of nucleic acid amplification along with an antibody-based assay (Guangxin et al., 2015). It uses a DNA-antibody conjugate as a bridge which links the immunoreaction with PCR reaction. This method has better specificity and 10^9 fold more sensitivity than conventional ELISA assay (Ruiyan & Huisheng, 2015; Chang et al., 2016). The latest advancements in this technique include better production of DNA-antibody conjugate and better readout methods. It also has broad range of applications in clinical diagnostics because it is an ultrasensitive protein detection assay (Chang et al., 2016). Several developed Nano-Immuno-PCR assays for disease diagnosis have been listed in the Table 6.

Conclusion

Nanomaterials offer a vast number of breakthroughs such as cost effective, lower risk to consumers and faster approach that will further enhance the clinical aspect of veterinary sciences in future and conceived that bacterial infections can be eliminated in the patient within minutes, instead of using treatment with antibiotics over a period of weeks. Nanotechnology has found its way into the food industry to improve food shelf life, safety and quality control. In coming years it can be expected that nanotechnology may practically apply in artificial creation of cells, tissues and organs. The artificial cells can be used in replacement of defective cells and organs, especially in metabolic disorders. Nanotechnologies have power to extent the modern molecular diagnostics to personalized medicine and therapeutics. Such techniques have

been used in the field of pathogen detection, DNA detection assay, biomarker discovery and cancer diagnosis. Nano medicine also has important role in future therapeutics as well as diagnostic assays. Although nanotechnologies have several applications and benefits, it is still in the early stages of its development and yet to apply throughout the world for routine diagnostics and therapeutics approaches.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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Table 6 Nano-Immuno PCR for detection of biological analytes.

Analyte	Nanoparticle	Detection limit	Reference
Diethyle phthalate DEP	GNP	4pg/liter	Ruiyan & Huisheng 2015
Aroclor 1248	GNP	2.55pg/liter	Guangxin et al., 2015
Tuberculosis Ag85B	GNP	90.9%	Netrapal et al., 2016
Alzheimer's disease Tau marker	GNP	Superior to ELISA	Stegurova et al., 2014
Nasopharyngeal carcinoma NPC	MWCNT	1:10,000,000	Ching et al., 2016
Hantaan Virus Nucleocapside	GNP	10fg/ml	Longyan et al., 2009
Hepatitis B surface Ag HBsAg	MNPs	320pg/ml	Wacker et al., 2007

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