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# Nanodiagnostics: leaving the research lab to enter the clinics?

**Abstract:** Nanotechnology has provided a plethora of valuable tools that can be applied for the detection of biomolecules and analytes relevant for diagnosis purposes – nanodiagnostics. This surging new field of molecular diagnostics has been revolutionizing laboratory procedures and providing new ways to assess disease biomarkers with increased sensitivity. While most of the reported nanodiagnostics systems are proof-of-concepts that demonstrate their efficacy in the lab, several nanodiagnostics platforms have already matured to a level that open the way for effective translation to the clinics. Nanodiagnostics platforms (e.g., gold nanoparticles containing systems) have been remarkably useful for the development of molecular diagnosis strategies for DNA/RNA detection and characterization, including systems suitable for point-of-care. How near are nanodiagnostics to go from the bench to the bedside?

**Keywords:** gold nanoparticles; molecular diagnostics; nanodiagnostics; nanotechnology; point-of-care.

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## Molecular nanodiagnostics

Nanotechnology has had a strong impact in biomedicine, notably in nanomedicine, particularly focusing on the diagnosis, treatment and prevention of disease, especially by enabling early disease detection and diagnosis, as well as precise and effective therapy [1]. Nanotechnology may be perceived as the use of tools and systems at the nanoscale. However, this description is rather crude and does not reflect the tremendous impact of nanotechnology

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in society. Despite the number of definitions that can be found for nanotechnology, that put forward by the US National Nanotechnology Initiative ([www.nano.gov](http://www.nano.gov)), later used by the US Environmental Protection Agency ([www.epa.gov](http://www.epa.gov)), requires that nanotechnology be applied when the tools/systems encompass: i) “research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of approximately 1–100 nanometer (nm) range in any direction; ii) creating and using structures, devices, and systems that have novel properties and functions as a result of their small and/or intermediate size; and iii) have the ability to control or manipulate on the atomic scale”. From this, it becomes clear that it is not just a question of downsizing the features of bulk materials but different, unique properties and manipulations ought to be present.

Nanodiagnostics can be described as the use of nanotechnology for diagnostics purposes. This includes, but is not limited to, single molecule manipulation and assessment, miniaturization of systems and platforms to make use of nanoscale properties derived from interactions between surfaces and biomolecules, etc. Nanodiagnostics has been evolving to meet the demand of clinical diagnostics for increased sensitivity and earlier detection of disease. The focus has been on the development of ever more sensitive transducing mechanisms to indicate that a diagnostically significant interaction occurs between analytes and the probing moiety. Most reports have put forward nanodevices for *in vitro* molecular assessment (e.g., detection and characterization of DNA and/or RNA; peptides and antibodies or antigens; small analytes), but several developments have also been made towards enhancing the diagnosis potential of *in vivo* platforms, usually coupled with existing imaging technology (magnetic resonance imaging, MRI; etc.).

The miniaturization possibilities coupled with the high sensitivity of nanodiagnostics platforms have prompted the development of devices capable of accurate molecular diagnostics at point-of-care (POC) [2].

It is commonly accepted that most clinical molecular diagnostics applications rely on the identification

of biomarker for disease characterization (e.g., proteins and nucleic acids). Most commonly, strategies directed at nucleic acid sensing, whether for detection of DNA/RNA or for selective characterization of target nucleotide sequences at single base mismatch resolution (e.g., single nucleotide polymorphism, SNP; mutations) are usually based on assessing the stability of the base pairing as a function of hybridization stringency [3]. When dealing with DNA, the gold standard is amplification via the polymerase chain reaction followed by fragment analysis and/or Sanger sequencing. As for RNA, the gold standard is commonly accepted to be RT-qPCR. When specifically addressing protein biosensing, commonly platforms include standard assembly concepts using antigens and antibodies for specific molecular recognition (e.g., immunoassays) that are coupled to the existing detection strategies, such as spectroscopy, fluorometry or electrochemistry [4–6]. A growing area of research is the use of aptamers that provide for analyte recognition that can be visualized via an intercalating agent or dye [7].

The detection and molecular characterization principles associated with nanodiagnostics platforms are basically the same, where the signal transduction is by means of a nanodevice or a nanoscale tool. These systems include micro and nanofluidics devices, microgravimetric and miniaturized piezoelectric transducers, nanotubes, but, perhaps the most diverse and widely reported are systems based on nanoparticles [8]. Nanoparticles (NPs) have brought a new dimension to biomolecular detection mainly by providing increased sensitivity at lower costs. Because most of these assays rely on canonical targets and widely used biomarkers, integration into nanoparticle-based diagnostic platforms has been forthright. One should mention that most of these nanodiagnostics platforms have been developed and optimized for the detection of pathogens [9] and cancer biomarkers [10].

There is a wide variety of nanoparticle-based applications towards nucleic acid detection and protein biomarker identification. However, detection and characterization of nucleic acid sequences using NPs systems has focused on three main types of nanoparticles: gold, silver and magnetic (iron oxide). In fact, noble metal nanoparticles have attracted particular interest for use in biomedicine, namely for low-cost high-sensitivity approaches for molecular recognition assays due to their unique physico-chemical properties, namely ease of synthesis and functionalization with DNA/RNA molecules, proteins or other biomolecules; and their nano-size scale provides for high surface-area-to-volume ratio, with great capability of interaction in the same scale of target biological molecules. This particular characteristic, i.e.,

the possibility to question the target biomolecule on a 1:1 scale, enhances the detection capability, which has led to new limits-of-detection when compared with standard methods. Because of this increase in sensitivity, most of the approaches relying on nanoparticle signal transduction are capable of decreasing consumption of reagents and decreasing the time required for analysis, which, together, greatly reduces the inherent costs. Several detection methods have been presented, i.e., colorimetric, fluorescence, mass spectrometry, electrochemical, and scattering. Nonetheless, despite current and novel concepts being reported each day, the vast majority of the proposed platforms have yet to be evaluated and screened for robustness under assay conditions, and most of these systems are still in pre-clinic with few commercially available products being translated into the clinic.

The ease of functionalization with oligonucleotides through a thiol bond [11, 12] – nanoprobe – make these nanoparticle-based systems extremely versatile and have, therefore, been applied in the specific detection of DNA/RNA targets based on hybridization protocols. The result of this hybridization may then be sensed making use of the NPs unique properties, i.e., optical absorption, fluorescence, Raman scattering, atomic or magnetic force and electrical conductivity. Nanoparticle-based nanodiagnostics platforms have mainly been developed towards the detection of biomarkers of genetic diseases, SNP genotyping and detection of pathogens' nucleic acids (bacteria and virus) with a detection limit in the fmol/L scale. Some reports claim even lower detection limits under strictly controlled conditions (e.g., amol/L), demonstrating the potential of these type of particles [13].

From the commercially proposed methods for bimolecular detection, colorimetric approaches are the most common, probably due to their simplicity, low costs and possibility of being made portable and, thus, susceptible for use at POC. Gold and silver nanoparticles are frequently used in colorimetric systems due to their high scattering in the visible region of the spectra. Nanoparticles stay in solution in the form of colloids, and present a localized surface plasmon resonance (LSPR) band that is dependent on the distance between nanoparticles or changes to the size or shape. Generally, colorimetric detection methods rely on the shift of the LSPR peak that is associated with aggregation, i.e., decrease of inter-particle distance. Two distinct approaches have been proposed to induce aggregation and hence the colorimetric signal: i) upon hybridization to the target molecule, the probe length induces approximation of the nanoparticle by means of the formation of a network of strands made

out of the probe and target (cross-linking approach); ii) aggregation may be induced through a modification of the dielectric medium around the NP (e.g., salt addition, pH, analyte) [12]. Despite AgNPs' higher coefficient of molar extinction when compared to AuNPs, functionalization with biomolecules is not as straightforward and AuNPs are used in most colorimetric approaches. The nanoparticle-based systems have been reported for direct detection of target biomolecule or, in the case of nucleic acids, following a step of PCR or isothermal amplification of the target.

The versatility of nanoparticles for nanodiagnostics is clearly reliant on their application in lateral flow assays (LFA) that allow straightforward molecular detection with great simplicity of use, allowing a low-cost, portable method, available for in-home diagnostics. In fact, LFAs are the most common commercially available POC diagnostic format, which can be used in a wide range of applications: detection of biomarkers (glucose, metabolic disorder, pregnancy, etc.), infectious agents, immunodiagnostics, small analytes and nucleic acids. Most of these applications rely on the direct recognition of the analyte by the capture probe that is usually bonded to a (gold) nanoparticle. The intensity of color provided by this nanomaterial makes it easy to detect against the white background of the pad. However, when considering LFAs for nucleic acid detection, the system generally relies on tagging the capture probe with streptavidin-biotin conjugations for detection of PCR amplicons, mutation and SNP discrimination, and identification of miRNAs. Application of these LFAs for the direct detection of unamplified DNA samples has also been reported [14, 15].

One of the main advantages of using metal nanoparticles in colorimetric systems is that they are capable of providing a comparable performance to that of fluorescence tags at a fraction of the cost and without the need for cumbersome apparatus. Integration of these detection strategies onto suitable platforms may, therefore, be made inexpensive to manufacture and suitable for portability [16, 17].

## Nanodiagnostics translation to the clinics

Nanodiagnostics have evolved from the basic laboratory concepts capable of detecting a biomolecule under controlled conditions to a series of prototypes that are on their way to laboratory validation. Nevertheless, the number of systems that have been validated is extremely reduced

when compared to the reports in the literature. In fact, only a few of these systems has made it to the pre-clinic or even the clinical stage.

Translation of nanotechnology research breakthroughs into health care services can be a hard job. Translational research involves the transfiguration of basic scientific research into clinical tools and/or systems capable suitable for application in the clinics, i.e., from bench (research laboratory level) to bedside (clinical level). This is not a straightforward task, as even the most promising results of basic research require rigorous independent screening and scrutiny before they can be effectively translated into the clinical lab and become a routine procedure. Many systems initiate this strenuous path but only a few make it to the bedside [18]. For a standard, traditional technology, successful translation to the clinics encompass the technology itself (advantages, efficiency, merit), coupled to the team innovation and funding. Where nanodiagnostics is concerned, due to its particular basis on nanotechnology and nanosized tools and systems, there are more issues to be taken into account. In fact, up to now, on either side of the Atlantic, there are no regulations implemented by the regulatory bodies that incorporate the use of nanodiagnostics features. This is partially due to the lack of concrete and standardized characterization protocols for the nanoscale items and products that can then be incorporated into the sensing platforms. Also, the understanding of the interactions of nanoscale materials with biological systems needs improvement. For example, size, composition, shape, and aggregation rate need to be considered on different biological levels, from cells to macromolecules. Some of the properties and mechanisms involved in bio-detection efficacy of these nanodiagnostics systems are poorly understood. Most of them have not been proven in simple biological samples conditions, let alone clinical sampling. Implementation of new methodologies that complement or substitute conventional biodetection and biosensing methods requires strict compliance with the existing detailed well-established guidelines and specifications for the specific purpose. For example, one of the recommendations put forward by the Organization for Economic Cooperation and Development (OECD) is that laboratories reporting genetic diagnosis "should be accredited or hold an equivalent recognition" of good practice. In the case of most of the nanodiagnostics platforms that address molecular detection and characterization, including genetic diagnosis, the laboratory that puts these systems into practice must ensure that the service or product are reliable, safe and error proof [19, 20]. Usually this effort is performed against robust standard

techniques that are considered the gold standard for a given detection item. At first this should not constitute a problem. However, nanodiagnostics tools are consistently breaking new barriers in terms of sensitivity, which sometimes may translate into “false-positives” when compared directly to the gold standard. How to circumvent this? And what is the clinical relevance of a lower than previously detected amount of a given analyte? How does one get such a system or platform correctly validated? These are some of the concerns that occupy those currently responsible for drafting and putting forward the guidelines ruling the nanodevices and diagnostics devices based on nanotechnology.

The technology behind the nanodiagnostics concepts has reached the optimal level of maturity to be translated into clinics. From the laboratory research point of view the time has come to translate all these promising platforms into clinics. In fact, some of them have already done so. For example, Nanosphere™ is a US company with several FDA approved diagnostic products that use gold nanoparticles as tags for labeling a wide range of probes suitable for molecular recognition. One of its products is Verigene®, which uses a parallel approach based on gold nanoprobe for the detection of several pathogens in a single assay step. Several cartridges are available for specific detection of pathogens and other genetic disorders. The technology is based on the microarray approach described by Storhoff and co-workers that does not require any previous step of DNA amplification [21]. The system depends on automated machinery for sample preparation and processing that is incorporated with the array and the readout system for data analysis. The available products include clinical microbiology tests (e.g., respiratory virus, Gram-positive blood culture, *Clostridium difficile*), and the thrombophilia test (F5, F2, MTHFR) and drug metabolism tests (Warfarin, CYP2C19) (<http://www.nanosphere.us/products>). The system advertises a limit of detection of as little as 50 fM of DNA with single base mismatch recognition that is suitable for mutation and/or SNP discrimination; sensitivity and specificity vary from product to product but are generally above 90% when compared to the gold standard techniques. Another example is the Magtration® Technology commercialized by Precision System Science Co., Ltd [22]. This platform makes use of magnetic nanoparticles to separate the target biomolecules of interest from complex media. Similarly to this one, Chemicell GmbH provides products for detection and separation of nucleic acids from biological fluids and media with extremely high purity, which optimize downstream application via traditional molecular biology approaches [23].

## Final remarks

There are a plethora of robust systems reported for the detection and characterization of biomolecules of interest to the clinician that make use of nanoscale tools, systems and devices. Most of these systems have been optimized to work in the research laboratory setting and are now mature enough to make their way into the clinics. Nanodiagnostics will definitely revolutionize the way we perceive molecular diagnostics, be it by the enhancement to sensitivity without loss in specificity when compare to traditional techniques, or by the lowering the amount of sample and reagents required to perform analysis, which in turn result in lower costs of operation. The proposed systems may also be miniaturized and used for multiparallel testing, thus enabling portability and automation that may, eventually, reduce the need for specialized personnel. These features make nanodiagnostics platforms optimal for use at POC. Whilst it is clear that nanodiagnostics has pushed the boundaries in biomolecule assays, translation to the clinic has still not been fully accomplished. In fact, only now are the advantages to the clinicians becoming clearer and the implementation still lacks the mandatory validation and performance evaluation. This is why, the diagnosis community, should begin to start focusing on these new platforms so as to reduce the time to the market by making use of the knowledge acquired thus far with other molecular assays.

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