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PERSPECTIVE

Patients, Here Comes More Nanotechnology

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ABSTRACT: **We describe the current difference in reporting the performance of nanotechnology diagnostic devices between technologists and clinicians. This perspective specifies the "metrics" used to evaluate these devices and describes strategies to bridge the gap between these two communities in order to accelerate the translation from academic bench to the clinic. We use two recently published *ACS Nano* articles to highlight the evaluation of silicon nanowire and surface-enhanced Raman spectroscopy-breath diagnostic tests for patients afflicted with cancer and asthma. These studies represent some of the earliest studies of emerging nanotechnology devices utilizing clinical parameters to assess performance.**

Throughout time, doctors have aimed to determine appropriate treatments for patients based on symptoms such as a fever, cough, or fatigue. In the last 30 years, researchers have started to focus on developing technologies that can provide molecular precision in determining the cause of a disease. This transition enables physicians to manage patient care more appropriately based on objective data. This approach has fueled the development of immunoassays to identify distinct protein biomarkers that are unique to a disease, as well as polymerase chain reaction to detect host genetic variabilities or human pathogens. However, despite contributions to improved patient outcomes, long turnaround times and the need for expensive reagents/instruments have limited the accessibility of these technologies to a broader pool of patients. Nanotechnology is a promising avenue to overcome some of these issues and will play a significant role in developing technologies to improve patient diagnoses.

WHERE ARE WE WITH NANOTECHNOLOGY-BASED DIAGNOSTICS?

Using nanoparticles to improve diagnostic devices was considered long before nanotechnology became popular. For example, gold nanoparticles were employed in lateral flow immunoassays (better known as a "dipstick") as early as the 1960s. The dipstick is a widely used form of capillary-driven diagnostics for biological samples, where an antigen interacts with immobilized primary antibodies and antibody-coated gold nanoparticle labels, to provide a red line indicating a positive result. Such a simple diagnostic device has leveraged nanotechnology for diagnosing pregnancy, infectious pathogens, as well as diabetic and cardiovascular diseases. Similarly, over the last 15 years, the research community has focused on incorporating nanoparticles in diagnostic assays or devices.[1−5] Diagnostics can exploit many desirable properties of nanotechnology including tunable optical, electrical, and magnetic properties. Gold nanoparticles, for example, are used in dipstick tests because of their high molar absorptivity coefficient contributing to an intense red color. Iron oxide nanoparticles are also used in many bead-based assays, as their magnetic properties simplify the purification and isolation of biomarkers.

> Nanotechnology offers diagnostics many desirable properties, such as tunable optical, electrical, and magnetic properties.

Due to the broad applications of nanoparticles in diagnostics and other medical applications, methods to synthesize and to characterize a variety of nanoparticle types, including semi-

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Table 1. Commonly Used Metrics and Terminologies for Clinical Assessment of Diagnostic Technologies[a]

term	description
true positive	a positive test result given by the diagnostic assay under evaluation that matches that of the reference standard
true negative	a negative test result given by the diagnostic assay under evaluation that matches that of the reference standard
false positive	a positive test result given by the diagnostic assay under evaluation that does not match that of the reference standard
false negative	a negative test result given by the diagnostic assay under evaluation that does not match that of the reference standard
sensitivity	$\text{sensitivity} = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}}$ — the predicted percent of true positives among all positive test results obtained by the reference standard
specificity	$\text{specificity} = \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}}$ — the predicted percent of true negatives among all negative test results obtained by the reference standard
positive predictive value	$\text{positive predictive value} = \frac{\text{true positives}}{\text{true positives} + \text{false positives}}$ — the predicted percent of true positives among all positive test results obtained by the diagnostic assay under evaluation
negative predictive value	$\text{negative predictive value} = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}}$ — the predicted percent of true negatives among all negative test results obtained by the diagnostic assay under evaluation
positive likelihood ratio	$\text{positive likelihood ratio} = \frac{\text{sensitivity}}{1 - \text{specificity}}$ — the odds of the test under evaluation to produce a positive test result when the disease is present *versus* a positive test result when the disease is absent
negative likelihood ratio	$\text{negative likelihood ratio} = \frac{1 - \text{sensitivity}}{\text{specificity}}$ — the odds of the test under evaluation to produce a positive test result when the disease is absent *versus* a negative test result when the disease is absent
receiver operator characteristic curves	the curve produced when true positives on the *y*-axis are compared against false positives on the *x*-axis, for a range of cutoff values for the diagnostic test under evaluation

[a]For in-depth reading on these metrics, please see refs 9, 14, and 15.

conductor, organic nanoparticles, and other metal nanoparticles, have been developed.[6] Tools that are aimed at measuring the properties and functions of these nanoparticles have also been developed and applied. Additionally, strategies to modify the nanoparticles' surfaces to enhance their water solubility and to coat them with nucleic acids, proteins, and other molecules to capture target biomarkers have been optimized for a number of applications.[7] These research activities have created a foundation for the use of nanoparticles as important building blocks to engineer diagnostic technologies. As a result, nanoparticles are used as colorimetric, fluorescent, magnetic, or thermal probes to detect biological molecules and can be incorporated into larger metal structures or polymeric beads to create barcoded platforms for the simultaneous detection of multiple biological molecules.

CLINICAL EVALUATION OF NANOTECHNOLOGY-BASED DIAGNOSTICS

Most researchers who are developing nanotechnology-based diagnostics come from engineering, chemistry, physics, or material sciences backgrounds.[8] When a diagnostic device is built, one typically determines the limit of detection and linear dynamic range of the device—all standard metrics used by engineers and physical scientists. In these experiments, the analyte to be detected is serially diluted, and the device measures the signal at each concentration. This is expressed as a graph of concentration *versus* signal, where the limit of detection is defined as three standard deviations above the mean background signal. The linear dynamic range is defined as the concentration range where the signal responds linearly to changes in concentration, and this range becomes an important metric for quantification of biological molecules. These analyses are often carried out on samples spiked in buffer, blood, plasma, urine, and other biological fluids. Performance characteristics of these assays are then reported. Although these analytical characteristics are fundamental in the development of new diagnostic assays, additional metrics are needed for clinical implementation. Commonly used terminologies, definitions, and equations related to clinical evaluation are listed in Table 1.[9] Currently, "technology" developers within academia rarely use clinical metrics or samples to evaluate the performance of diagnostic tests. In contrast to spiked samples, which are often used as a substitute for clinical samples, biological samples are complex and contain different types of molecules that can adversely influence the performance of the diagnostic assay under evaluation. This complexity makes it extremely difficult to recapitulate actual patient samples using spiked samples. By analyzing the performance of the device with patient samples, the researcher can obtain enough information to make the appropriate engineering changes to ensure adequate performance when used on patients. In our opinion, to expedite clinical translation, these "technology" developers at the academic level need to start using clinical samples and nomenclature to evaluate and to describe the performance of diagnostic tests.

EXAMPLES OF CLINICAL ADVANCEMENT FOR BREATH TESTS

ACS Nano has recently published a number of manuscripts that have demonstrated the successful use of nanotechnology-based diagnostics systems on clinical samples.[10,11] These studies include the use of silicon nanowire sensors to diagnose patients with gastric cancer, lung cancer, and asthma (as described by Shehada *et al.*)[12] as well as the use of surface-enhanced Raman scattering (as described by Chen *et al.*)[13] to distinguish between early and advanced gastric cancer. In both studies, the authors used the patient's exhaled breath to make the diagnosis, a method that is noninvasive and simple to conduct. The breath was analyzed for volatile organic compounds (VOCs), where the composition of the VOCs is used as a "breath-print" to differentiate between patients with and without cancer at different stages. Both studies shared similar approaches, where the surfaces of their sensors were chemically modified to increase the interaction with VOCs. When the patient breathes into the device, the VOCs bind to the sensor's surfaces and

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change the electrical signal or Raman signature. The generated signals are then deconvolved and compared between patient cohorts. The study by Shehada *et al.* included 374 subjects and achieved an 80% accuracy in differentiating between patients with and without cancer or asthma. Chen *et al.* tested 200 patient samples and achieved >83% clinical sensitivity and 92% clinical specificity for patients with early and late gastric cancer. These two reports demonstrate the clinical feasibility of emerging technologies through the use of patient samples and illustrate their diagnostic accuracy levels in comparison to reference tests.

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WHERE SHOULD THE CLINICAL TESTING OCCUR?

The implementation of novel diagnostic tests in the clinical setting is a lengthy process that involves (i) research and development, (ii) laboratory validation and evaluation, (iii) clinical evaluation and implementation, and (iv) ongoing proficiency testing. The depth and robustness of clinical evaluations are highly variable. In comparing new technology with the gold standard technology, new diagnostic tests must offer some advantage over established methods, whether it is in clinical sensitivity, clinical specificity, turnaround time, or cost.

It remains a challenge to understand where in the development cycle the clinical analysis of an emerging technology should occur. There are currently two research silos in the development of diagnostic technologies. The first is the "technology" developers, where a study is considered to be complete when researchers have characterized the analytical performance of the diagnostic device. The second is the "clinical" evaluators, where research activities of the diagnostic device occur after a company has built a black-box system. This community is mostly focused on clinical evaluation of the technology. In between these two research communities is the "entrepreneurial" community, where companies are started based on the technology developer's concept and analytical data. Founding a company can be risky, however, as the performance claims made by the technology community may not hold up when applied to patient samples.[6] Connecting to clinical needs and conducting a small amount of "clinical" testing early on in the development cycle can help predict clinical applicability of the device and guide the engineering process. Few published studies have combined the efforts of both the "technology" and "clinical" communities. Having these two communities work together early in the development process is important and can be critical to the successful translation of the technology.

After these issues have been addressed, the next path to translation will involve the identification of larger partners for full clinical evaluation. These partners are generally diagnostic laboratories in large tertiary care centers, reference centers, or other centers of excellence in a given diagnostic sphere. The clinical impact (or lack thereof) for some assays has been more extensively studied (*e.g.*, prostate specific antigen or PSA) than others (*e.g.*, the utility of 16S ribosomal sequencing in culture-negative sterile fluids). In all evaluations, the new technology has to be compared to the current gold standard techniques. Typically, this process involves evaluating clinical sensitivity, clinical specificity, diagnostic accuracy, turnaround times, and operational feasibility in the clinical setting to measure the clinical effectiveness of diagnostic tests and to determine a patient's prospective status. Other issues, such as downtimes due to instrument or reagent issues, complex/time-consuming steps, long turnaround times, or even incompatibilities with laboratory workflow need to be considered. Hence, a new test may not ultimately succeed, despite promising analytical performance characteristics.

CONCLUSION AND OUTLOOK

The nanotechnology community has made significant strides in the development of diagnostic devices. Unlike *in vivo* applications of nanoparticles, diagnostic devices are unlikely to have the stigma of nanoparticle toxicity since the analysis is done outside the body. This distinction would likely ensure that nanotechnology-based diagnostic devices advance more rapidly for clinical use. Many of these technologies remain at the academic stage and analytical performance is typically validated with spiked samples to convey diagnostic success. Here, we suggest a move toward clinical testing during the technology-development phase. Early testing of real samples will help engineer the device and should lead to more likely and more rapid translation of the technology.

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Notes

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