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Challenges of the nano-bio interface in lateral flow and dipstick immunoassays

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Abstract

Lateral flow assays (LFAs) are highly attractive for point of care diagnostics for infectious disease, food safety, and many other medical uses. The unique optical, electronic and chemical properties that arise from the nanostructured and material characteristics of nanoparticles provide an opportunity to increase LFA sensitivity and impart novel capabilities. However, interfacing to nanomaterials in complex biological environments is challenging and can result in undesirable side effects such as non-specific adsorption, protein denaturation, and steric hindrance. These issues are even more acute in LFAs, where there are many different types of inorganic-biological interfaces, often of complex nature. Therefore, the unique properties of nanomaterials for LFAs must be exploited in a way that addresses these interface challenges.

Keywords

Nano-bio interfaces; protein coronas; rapid diagnostics; paper-based assays

The need for lateral flow diagnostics

Epidemic outbreaks are a major global health threat, and the frequency of outbreaks has been steadily increasing.[1] Diagnosis is a critical step in confining and treating infected patients. There are several approaches for detecting viruses in patient samples, ranging from detecting the virus itself to the antibodies developed in the immune response [2]. Methods for diagnosis range in achievable specificity and sensitivity, where each approach has advantages and disadvantages. **Lateral flow assays (LFAs**, see Glossary) have been promising as point of care (POC) devices because they are easy to use, robust, inexpensive,

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and implementable in rugged environments. [3] LFAs consist of paper strips to which a biological sample is added, and the fluid wicks through, resulting in two colored lines for a positive test, or one for a negative test. Readout relies on formation of a sandwich immunoassay when a biomarker is present, which is visible to the naked eye because of the accumulation of gold **nanoparticle (NP)**-antibody conjugates at the test line, though other nanomaterials outside of gold NPs have also been utilized such as dye-infused beads.[4]

Because of their simplicity and rugged format, LFAs have been used for many diverse applications not just including infectious disease, but also water and food safety, [5] routine clinical tests, and food allergy testing [6]. Thus, they have made a significant impact on public health. [7] There are many commercially available LFAs, some of which are summarized in Table 1. Available tests are primarily for testing serology by detecting IgG, IgM, or IgA antibodies produced by the immune system in response to pathogens (i.e., serological assays), and use a predominantly gold NP colorimetric readout. However, rapid tests can also detect metabolites (as in the case of drug testing) or the protein biomarkers themselves (i.e., direct assays).

Lateral flow diagnostics are enhanced by nanotechnology

LFAs rely on different modes of detection (Fig. 1), where readout can be optical-colorimetric, fluorescent, surface enhanced Raman spectroscopy (SERS), electrochemical, or biochemical. [8, 9] The species on the label antibody is often a gold nanoparticle due to its strong optical absorption. Other labels include dye-loaded polymeric beads, or fluorescent beads or quantum dots, magnetic nanoparticles, and carbon-based nanoparticles. [10] The combination of nanotechnology with point of care diagnostics has resulted in significant advances in device technology. [11–13] Nanomaterials possess unique material and size dependent properties that can enhance LFA detection by optical as well as fluorescent, spectroscopic, and electrochemical readouts. These advances can help create lower-cost, higher sensitivity, and field-forward devices.

However, the intersection of nanotechnology with biology also creates numerous challenges. Furthermore, the simplicity of LFA operation makes them appear deceptively straightforward to construct. A device that uses a simplified format of the LFAs is the dipstick assay, where the conjugate pad and sample pad are substituted with a solution into which the nitrocellulose with the wick is immersed. This eliminates the need to dry down the NP-biomolecule conjugate. Unfortunately, LFAs and the related **dipstick** immunoassays suffer numerous interface issues such as non-specific adsorption of proteins and gold NPs to the test substrate or test materials. These surface effects can have negative ramifications such as ambiguous readouts and reduction in sensitivity. Despite their importance, interface issues are often overlooked and underappreciated. While any inorganic-biological interface can have undesirable side effects, these cannot be ignored for nanomaterials because of their high surface to volume ratios. Solving interface issues can be tedious and/or viewed as having limited innovative aspects, so often they are not published in the literature.

These interface issues have long plagued the nanomedicine and nanobiotechnology communities, which have faced complications that result from putting nano- and micro-scale

inorganic materials in contact with biology. These complications include surface fouling, corrosion, and protein denaturation. For LFAs and dipstick immunoassays, these complications are amplified because there are two biological-inorganic interfaces, one for the NP and another for the nitrocellulose substrate. However, these surface effects can result in either false positives or false negatives, which can have life or death consequences.

Interface issues have been problematic in other nanotechnology fields such as cancer nanomedicine. Non-specific protein adsorption and **protein corona** formation (see Box 1) have been shown to reduce the specificity of ligand-cell receptor interactions, deteriorate the carrier efficacy, or cause undesirable biodistribution. While interface issues had been one of the biggest barriers for cancer nanomedicine, advances in recent years have promoted clinical applications of nanomaterials. Thus, these advances can help improve the development of LFAs. Despite the progress in nano-bio interface issues in cancer nanomedicine, they have not been extensively discussed for dipstick and lateral flow immunoassays. Therefore, this Review seeks to highlight the importance of nano-bio interface issues in point of care immunoassays, and what can be learned from other areas of nanomedicine where interface issues have been successfully overcome.

Box 1

Protein coronas

Protein coronas are clouds of weakly bound proteins that form around nanoparticles (NPs) when they are introduced into biological fluids. The protein corona, and not the NPs themselves or the NP surface chemistry, is known to be the most important factor in biological processes such as cell uptake and cell association. Understanding and manipulating protein coronas has been challenging, though concerted efforts in the field have resulted in major progress. Protein corona properties such as size, composition, etc. are difficult to measure because coronas are held together by non-covalent bonds. Predicting what corona will form has remained elusive because coronas are complex and heterogeneous. However, advances in proteomics of coronas has allowed insight as to what proteins tend to be in coronas and how they influence some biological process such as cell association[55]. Additionally, corona composition and size evolves with time, and depends on the history of the sample. There have been many attempts to make NP surfaces that are completely non-fouling, which have met sporadic or limited success. However, there has been some progress with zwitterionic molecules. The composition of the protein corona that forms around a NP differs for every biological fluid (serum vs. blood vs. urine vs. saliva), though coronas have been characterized predominantly for human serum, cell media, and blood.

Interactions at the nano-bio interface in lateral flow immunoassays

Several different interface issues in LFAs can affect the central phenomenon of antibody-antigen binding in the LFA. Often sandwich immunoassay formation is idealized as a single antigen binding cleanly to an antibody on a flat surface and another on a NP (Fig. 2a). However, undesirable interface issues can occur (Fig. 2b), including non-specific adsorption

and steric hindrance on both the NP and the nitrocellulose. These can be categorized into different types of interface interactions.

Interface issues for the NP-antibody conjugates

Like with any NP-biomolecule conjugate, the ability of the antibody (Ab) to bind to its target antigen can be diminished because of its attachment to a NP.[14] Most often this is manifested by a change in binding affinity, K . Studies of immobilized DNA have shown that surface immobilization can either increase or decrease the DNA's affinity for its complement, changing K over 29 orders of magnitude [15, 16]. The Ab can denature due to perturbing effects of the NP and its surface coating ligand, preventing biomarker binding. The Ab can be potentially oriented incorrectly on the NP surface, where its binding epitopes for the biomarker are obscured, lowering or even preventing target binding. Finally, the surface ligands on the NP surface can sterically obscure the binding epitopes of the Ab.

Furthermore, the interaction of the NP-Ab with its environment can also impact Ab-antigen binding. Samples added to LFAs contain complex mixtures of proteins and small molecules in the presence of which the binding event must take place. Often biological samples for POC assays are not cleaned up, and even if they are, proteins and salts can still be present at high concentrations. When NPs are introduced into biological fluids, a protein corona forms around them, where the proteins non-covalently adsorb to the NP surface, forming a weakly bound "cloud" (Box 1). [17] [18] Often protein coronas are studied for cancer delivery agents, [19] but they are also present in LFA devices and undoubtedly influence antigen-antibody interactions.

The properties of the corona that forms around a NP is influenced by NP size, material, and shape, and strongly influenced by NP surface chemistry.[20] Unfortunately the molecules used to passivate NP surfaces are typically a trade secret for commercially available NPs, which makes it difficult for end users to optimize the system for Ab conjugation.

Protein corona formation is rapid: proteins adsorb to the NP surface within seconds.[21] In typical LFAs the fluid front takes minutes to reach the test line, and often the test is allowed additional time to "develop," so NP-antibody antigen conjugates most certainly have a protein corona. To further complicate matters, the biological fluids for LFAs are diverse, and can be blood, serum, urine, saliva, or others, all of which will have very different compositions, and thus will all form different protein coronas.

Interface issues for the immobilized antibodies

The interface issues for the NP-Ab conjugate are mirrored for the immobilized Ab. Because paper has several unique properties as a substrate, paper analytical devices and bioactive paper have had a surge of interest. There is a major advantage to immobilizing reactions on paper as opposed to having them in solution as it eliminates the need to transport fluids and a cold chain. Due to their chip-based format, paper-based devices are easily miniaturized, can be manufactured at scale, and are often considered to be the most widely deployed microfluidic devices. [22] There has been extensive work studying biomolecule conjugation to cellulose for paper supported assays [23]. Antibody immobilization onto nitrocellulose is most often achieved simply by spotting it down, where the Ab adheres by hydrophobic

interactions. Alternative strategies include chemical conjugation to the nitrocellulose or to streptavidin have also been used successfully. [24] Generally, immobilization onto paper can result in different target affinities. Again, antibody behavior on paper can differ significantly from solution or ELISA. Paper is a much more complex substrate compared to flat glass, polystyrene, and metal surfaces that are typically used for ELISA, surface plasmon resonance (SPR) sensing, and other assays. Its porosity provides the driving force for the capillary flow, but this means that net surface area is high, amplifying interface effects. Like with the NP-Ab conjugate, the nitrocellulose can also have a “protein corona” or surface adsorption of the proteins from the biological fluid. Additionally, biomolecules can be trapped inside the pores and decrease target binding efficiency. Typically these adsorption issues are mitigated by membrane blocking *via* adsorption of other proteins such as albumin, casein, and other proteins. However, blocking paper can come at the cost of reducing assay signal and thus sensitivity. [25]

Antibody-biomarker interactions

The antibodies on the NP and test line must be able to bind to the target. For a given disease, the antibodies must be able to recognize targets even when there are differences due to strain and serotype. Furthermore, antigens from different geographic areas can present different epitopes or glycosylation, making it difficult to have one specific antibody for each disease, but an antibody cocktail can be utilized to ensure binding to all variations. To complicate matters, viruses evolve during an outbreak, which can result in the antibodies in the test strip to become less effective at detecting the antigen, or cross reactivity with closely related viruses.

For sandwich formation, the antibodies on the NP and nitrocellulose must be able to bind to the antigen of interest simultaneously. Thus, they must work in pairs, so the antibody on the NP cannot bind to the same epitope as the nitrocellulose antibody or interfere with each other. Therefore, even though a target antigen can have multiple antibodies that recognize it, not all of them will work in pairs.

In reality, antibodies do not have mutually exclusive targets, and can bind to multiple species, especially if biomarkers are closely related. Cross reactivity can be minimized by increasing stringency in screening, but because the nature of epidemics, the target antigen can evolve, resulting in unanticipated cross-reactivity. For diagnostics to be useful, they must be multiplexed, [26] which can become complicated by cross-reactivity within a device.

Finally, patient samples are more complicated than pure solutions. Testing in the lab is often performed on purified protein in buffer or spiked into human serum, which is not nearly as complex as samples obtained from patients in the field. Sample preparation can also greatly influence assay functionality and accuracy, and while minimization of processing steps is more convenient, “raw” samples can be much more variable. These factors can contribute to assay variability.

Overall device

The overall device design requirements are that it needs to be simple to use, where the result can be readable by eye and easily interpreted by the user. This places constraints on the

signal intensity at the test line, as it must be detectable by eye. The colorimetric intensity in a LFA is a function of multiple parameters (Fig. 3). Some of these parameters are straightforward and predictable, such as antigen concentration, and others such as NP size have been measured. [27] In addition, the number of antibodies on the NP surface (i.e., antibody coverage) is also a critical parameter. However, many of the parameters are not intuitively obvious, such as Ab coverage and NP surface chemistry. Nevertheless, this equation serves as a guide for considering all of the NP-Ab and other factors that are at play in generating a test line signal.

The LFA also presents challenges in that target recognition must occur under fluid flow, so the binding event does not occur at equilibrium. [28] Finally, the device operation is subjected to environmental conditions where temperature, humidity, and lighting conditions differ greatly from the laboratory.

Design considerations for LFAs

Because LFAs and sandwich immunoassays are now a mature technology, there are numerous industry-written guides and workshops that discuss best practices for designing and manufacturing LFAs with high sensitivity and specificity, can be scaled up, and are appropriate for consumer use. [29] However, the interface challenges mentioned above still remain. Going forward, what is the best strategy for making LFAs that rely on nanotechnology? These issues need to be considered for the next outbreak that emerges. Even though LFA technology is not new, we still must consider issues that may present design constraints for each new or re-emerging disease (Fig. 4). Factors include the behavior of the disease itself, such as how infectious it is and the nature of the symptoms and outcomes. Also, characteristics of the endemic areas need to be considered, such as whether it is urban or rural, available health care, and if similar diseases are currently co-circulating. Then, the technical aspects of detection need to be considered, such as the identity of the biomarker that will be detected, what concentration it exists at, and how distinct it is from biomarkers of similarly related diseases. In addition, the availability of bioreagents to detect the biomarker of interest is critical. Generating and selecting antibodies, and their mass production requires 16–24 months, [30] [31] which is much longer than the critical initial period when an outbreak is emerging. Additionally, the nature of the patient sample can influence test design, as sample collection issues are different for urine collection vs. blood. Finally, readout modality should be chosen to optimize sensitivity, or whether a reader is required, or if readout by eye is important. Making a point of care device is inherently a multidisciplinary effort, as it involves technical expertise in immunology, molecular biology, infectious disease, materials science, fluid mechanics, and manufacturing. In addition, critical aspects of device usage go beyond the technical, as human interactions, socioeconomic issues, and public health aspects can strongly influence device design. For success, challenges in each of the technical fields must be fully appreciated by the others.

The World Health Organization (WHO) has issued criteria under the acronym ASSURED (see Box 2) which are guidelines that point of care diagnostics must meet in order to be useful in the developing world. Even though the needs for resource-poor settings share common characteristics, such being low-cost and rugged, constraints will differ for every

outbreak. For example, in the 2014 Ebola outbreak, the high contagion level and human-to-human transmission emphasized the need for a differential multiplexed diagnostic that could diagnose Ebola and also confirm that symptoms were not due to another co-circulating disease, such as malaria. In addition, sample handling can also place constraints on the design of how biological fluids are introduced to minimize contamination of the health care worker administering or assay readout.

Box 2

World Health Organization ASSURED criteria

Disease diagnostics are the most useful for populations in the developing world, where disease burden is the highest. Traditional lab tests are not feasible because they require infrastructure typically not available in these resource-poor settings. Consequently, the World Health Organization has issued guiding criteria for diagnostics, with the acronym ASSURED:

Affordable

Sensitive

Specific

User-friendly

Rapid & Robust

Equipment-Free

Delivered

Devices that meet ASSURED criteria are amenable for developing countries and rugged environments where many infectious diseases are endemic.

Towards improved nanotechnology-enabled LFAs

To overcome interface issues requires an understanding of them. Fortunately, the field has made significant progress overcoming and adapting to nano-bio interface issues. For example, our understanding of surface modification strategies of nanoparticles is now highly sophisticated, which has facilitated the development of nanoparticle drug carriers or imaging agents for cancer therapy. [32] Clearly, a multidisciplinary approach is critical for overcoming these challenges. This means that material scientists must appreciate the complexity of the biological samples and the behavior of antibody-antigen binding. Likewise, immunologists and virologists must recognize the unique challenges of NP-biomolecule conjugates and NPs in biological environments.

Currently, information on NP functionalization for LFAs has been growing. Commercial NP sources such as Nanocomposix [33] are now publishing guides that discuss tips for using NPs in LFAs in an effort to bridge the gap. Handbooks and protocol chapters have been helpful in disseminating step by step recipes and best practices for making LFAs.[34]

However, one major impediment is that surface treatment of the NPs and the nitrocellulose/conjugate and surface pads in commercial assays are predominantly proprietary information. This greatly hinders troubleshooting, as NP surface chemistries and stabilization molecules are critical to the success of a paper-based immunoassay.

Thinking beyond best practices, it is also important to look towards new and emerging approaches. There have been some innovative ways to utilizing the unique properties of nanoparticles for LFAs, listed below:

Further ways to exploit nanotechnology

Even though NPs have been used in LFAs for decades, there are still many unique opportunities for exploiting their properties to improve LFA performance and also enhance their capabilities. For example, photothermal heating of the NPs [35] can be used to increase the signal of the test line, where a laser is used to excite the gold NPs at their SPR and increase image contrast by thermal detection. Signal enhancement has been achieved by enzymes such as horseradish peroxidase (HRP) [36], silver staining, [37] or even the use of multiple gold NPs binding species [38], all of which increase assay sensitivity. Induced NP aggregation has also been used to increase the signal or effect a color change. [39, 40]

NPs can be used for other modes of readouts besides visual imaging. Surface enhanced Raman spectroscopy (SERS) has been attractive for analyte detection because it can enhance the signal of molecules in its vicinity by several orders of magnitude.[41] For biological sensing and imaging, a format where the gold NP is decorated with a small molecule reporter, resulting in a “nanotag.” While this has been used for SERS imaging, it has recently been explored for LFAs.[42, 43] Superparamagnetic iron oxide nanoparticles (SPIONs) have also been investigated for a magnetic LFA readout [44].

Additionally, nanotechnology can enable new capabilities to assays. For example, test line colors have been traditionally only a single color, red, due to the size and shape gold NPs used in the assay. That NPs change their color with their shape and size has been well-studied, but only recently has it been used to enable spectral multiplexing by using multicolor test lines, as has been demonstrated for antibiotic detection using fluorescence of quantum dots [45], for Dengue/Yellow Fever/Ebola using visual readout of silver NPs,[46] and also dye-labeled cellulose particles for detection of Dengue/Chikungunya IgG/IgM. [47]

Exploiting protein coronas

As mentioned previously, protein corona formation is typically viewed as a hindrance (Box 1). Proteins in solution adsorb to the NP, obscuring ligands on the NP surface to bind to its target. For LFAs, the corona can potentially block the Ab from binding to the target antigen in the sandwich immunoassay. However, protein coronas do not produce only negative side effects, and it is now known that they can be exploited for their unique properties. Recently, the impact of the protein corona around NP-Ab in paper-based immunoassays was examined. [48] Anecdotally it is known that running strips in buffer solution often does not work because false test lines occur; this effect can be prevented by running strips in human serum. The NP-Ab in the serum forms a protein corona, and the corona is partially responsible for preventing non-specific adsorption to the test line, reducing false positives.

These corona proteins actually mediate the interaction between the NP-Ab antigen complex or the NP-Ab and the immobilized Ab in a favorable way.

Fluid flow manipulation

Approaches to increase sensitivity by manipulating the fluid flow have also been explored, some of which can be used to improve NP-Ab interaction with the antigen and test line. For example, isotachaphoresis,[49] an electrokinetic preconcentration/separation technique, can improve transport of target analytes to the LFA capture line, resulting in a dramatic increase in sensitivity. There have been also efforts to modify fluid flow by use of wax pillars or shaping of paperfluidic channels [50, 51]. However, this aspect of LFAs has great potential for innovation, and can be used in addition to other methods for improving performance and sensitivity via NP-Ab optimization. [28]

Quantitative descriptions of binding events

Finally, a molecular level understanding of the binding event and the surface effects and perturbations is critical for improving antigen binding, which is at the heart of the immunoassay. Even though Ab-antigen binding does not occur at equilibrium, binding affinity constants (K) are useful for comparing the behavior of different tests when optimizing the properties of the NP, Ab, and test strip. For example, Langmuir isotherms modified to include surface terms can be used to separate the impact of the surface modifications on antibody-antigen binding in planar and NP surfaces, and can also be applied to LFAs.[48, 52, 53]

Concluding remarks and perspectives for the future

In summary, LFA and paper-based assays have been proven to be useful as diagnostics, with tremendous potential to improve response to disease outbreaks. In addition, there have been many innovations in using them for many other applications in unique ways. Despite the fact that LFAs have been around for many years, they can continue to take advantage the unique capabilities of nanotechnology, as both fields are still evolving.[54] Consequently, we have to be cognizant of bio-interface issues as they continue to emerge. Many outstanding issues remain (see Outstanding Questions), but as with other biological fields that intersect with nanotechnology, understanding and overcoming interface issues has facilitated the success of innovative applications.

Outstanding Questions

- How can we properly exploit nanotechnology to enable robust and sensitive POC devices?
- How can we engineer optimal devices for the next epidemic outbreak in a timely manner?
- LFAs rely on nanoparticle-biomolecule conjugates. With the increasing number of technical advances in nanomaterial manufacturing, can scale up also be reached for nanoparticle-biomolecule conjugates?

- Can the rapid sample-to-answer time of LFAs be ultimately leveraged for epidemic control, such as making real-time maps of epidemic outbreaks based on actual patient data?
- Will competing technologies such as nucleic amplification techniques render LFAs obsolete? With the increasing innovation in LFAs, what characteristics of LFAs vs. nucleic acid devices complement or compete with each other?

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Glossary

Dipstick assay

a paper-based assay for detecting that is a simplified version of the lateral flow assay (LFA), which consists of a nitrocellulose strip which has immobilized antibodies that is put in contact with a solution containing the biological sample mixed in with the nanoparticle-antibody conjugates

Lateral flow assay (LFA)

a paper-based device for detecting the presence of a biomarker, antigen, or other analyte. A fluid is added to a sample pad and then wicks through a conjugate pad that has dried into it a nanoparticle-antibody conjugate or similar species that can specifically bind to the biomarker. The complex wicks through a nitrocellulose strip, which has immobilized antibodies or other binding species that capture the complex, providing the readout

Nanoparticle (NP)

a particle with dimensions in the range of 1–100s of nanometers

Protein corona

the “cloud” of proteins and other small molecules that forms around nanomaterials when they are introduced to biological fluids and physiological environments

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Trends

- Lateral flow assays (LFAs) for infectious disease, food safety, and many other applications have been enhanced by nanotechnology.
- However, interface effects in LFAs are much more complicated, which is problematic because POCs have to be robust, simple, easy to use.
- To fully utilize the unique properties of nanotechnology, these interface issues must be understood, controlled, and also leveraged.

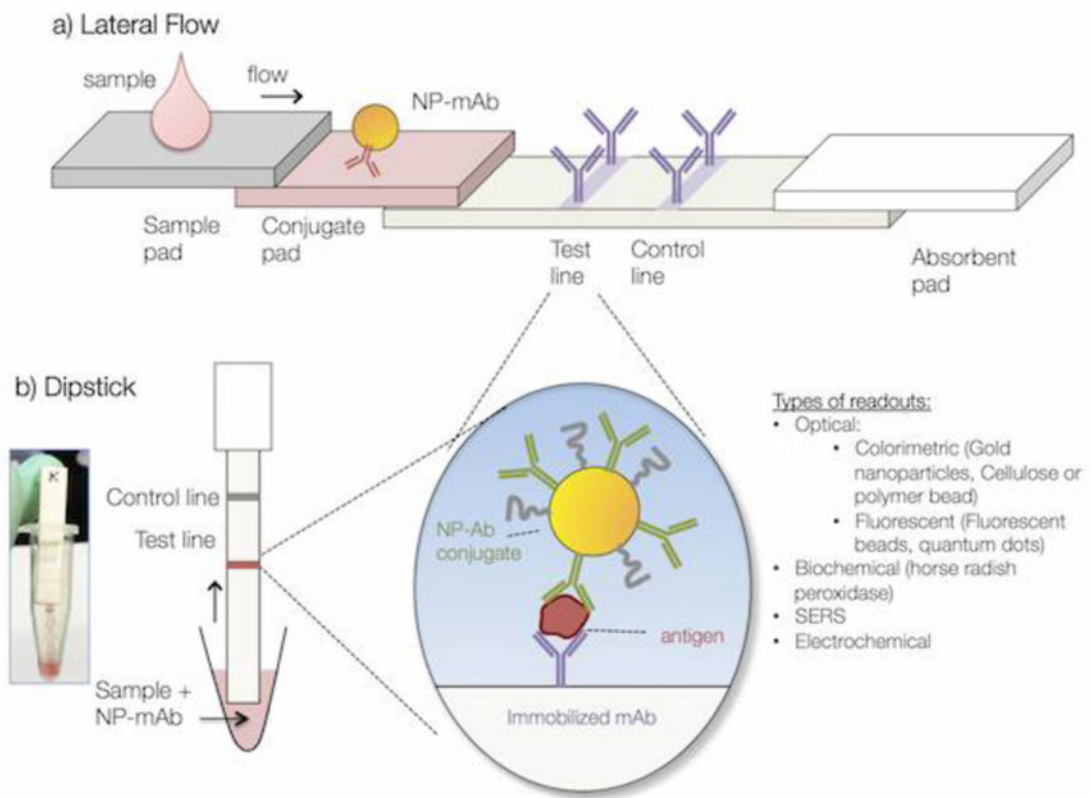


Figure 1.
Basic structure of a) an LFA and b) a dipstick immunoassay.

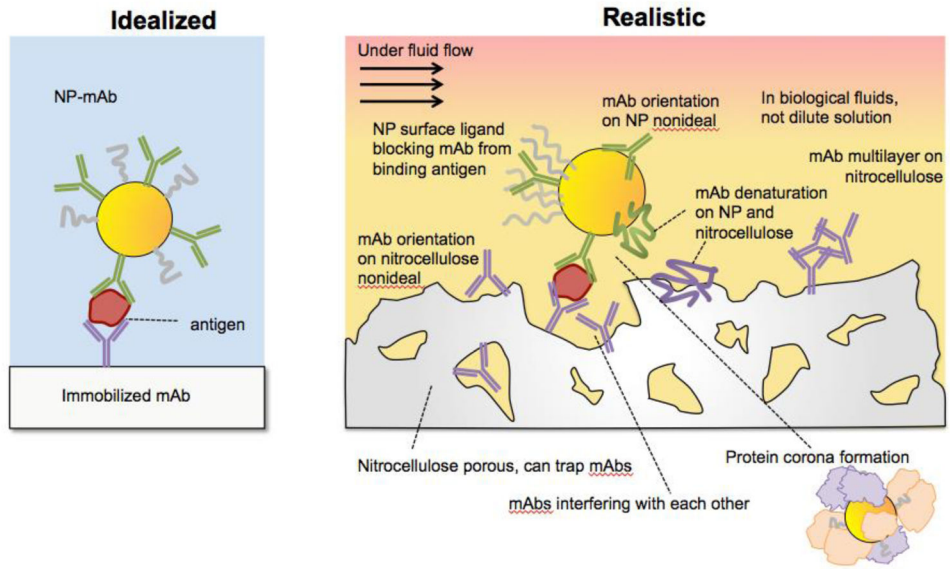


Figure 2. Idealized vs. realistic sandwich immunoassays that use nanoparticle antibody conjugates for sensing.

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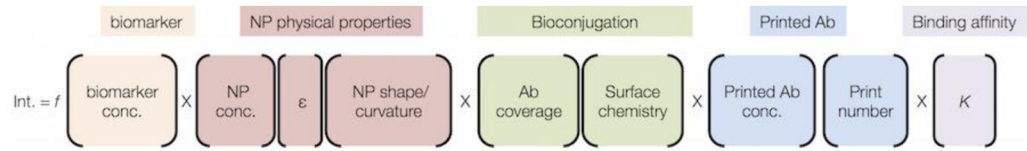


Figure 3.

The test line intensity of a LFA that uses a visual readout is a function (f) of multiple parameters, which are categorized by color.

Design considerations for rapid POC diagnostics

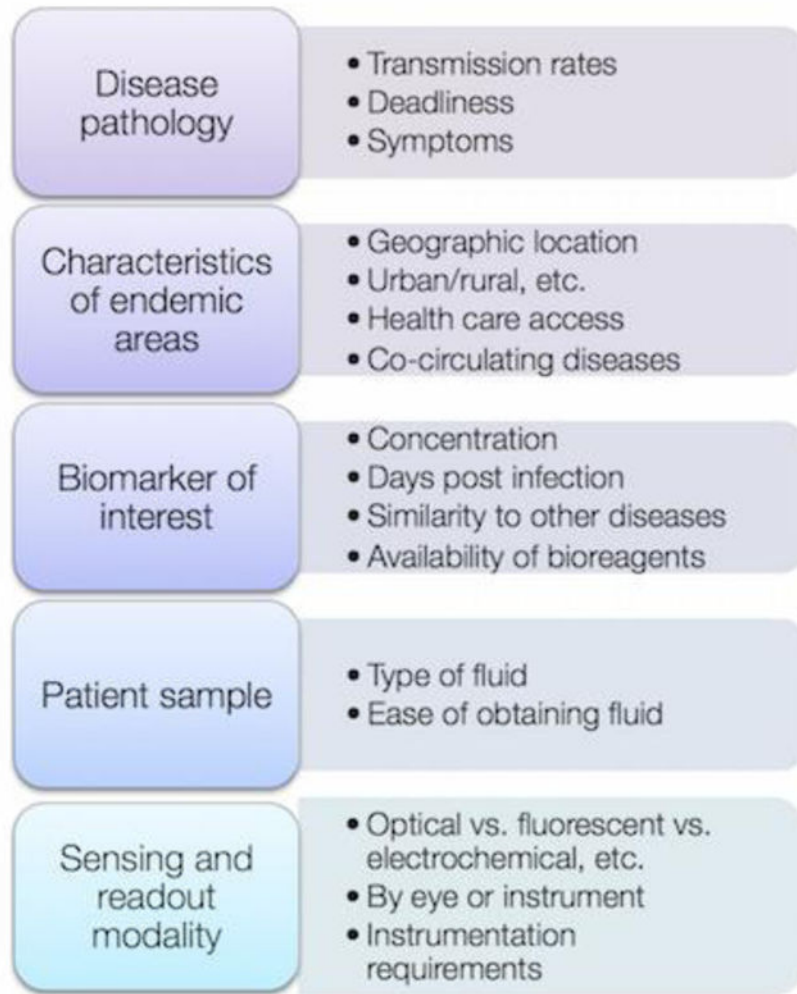


Figure 4. Design considerations for point of care diagnostics for infectious disease.

Table 1

Table of rapid tests for different types of biomarkers, their manufacturers, and the detection target.

pathogen	sources	Detection
Blood Borne Diseases/Infectious Diseases		
Cytomegalovirus	Biocan, Biogate Labs	IgG/IgM
Hepatitis A	Standard Diagnostics, Biogate Labs	IgG/IgM
Hepatitis B	Standard Diagnostics, Biocan, Cortez Diagnostics, Biogate Labs, Maternova, NTBIO	antigen, IgG/IgM
Hepatitis C	Standard Diagnostics, Orasure, Biocan, Cortez Diagnostics, Biogate Labs, Maternova, NTBIO	IgG/IgM
Herpes Simplex Virus	Biocan, Alere, Biogate Labs	IgG/IgM
HIV	Standard Diagnostics, InBios, Orasure, Biocan, Alere, biolytical, Biogate Labs, Maternova, NTBIO, Biopanda	antigen, IgG/IgM
Toxoplasma Gondii	Biocan, Biopanda	IgG/IgM
Infectious Disease		
Anti-EV71	Standard Diagnostics	IgM
Anti-Onchocerciasis	Standard Diagnostics	IgG
Chagas (Trypanosoma cruzi)	Standard Diagnostics, InBios, Biocan, Maternova	IgG/IgM/IgA
Chikungunya	Standard Diagnostics, Biocan, Maternova	IgG/IgM
Dengue	Standard Diagnostics, InBios, Biocan, Cortez Diagnostics, Access Bio, Biogate Labs, Maternova, Mediven, NTBIO, Biopanda, Focus Diagnostics	antigen, IgG/IgM, IgA
Ebola	Orasure, Biocan, Corgenix	antigen, IgG/IgM
Hanta	Standard Diagnostics	IgG/IgM/IgA
Japanese Encephalitis	Standard Diagnostics	IgM
Leishmaniasis	Standard Diagnostics, InBios, Biocan, Cortez Diagnostics	IgG/IgM
Leptospira	Standard Diagnostics, Biocan	IgG/IgM
Lymphatic Filariasis	Standard Diagnostics, Biocan	IgG/IgM
Malaria	Standard Diagnostics, Biocan, Alere, Cortez Diagnostics, Access Bio, Biogate Labs, Maternova, NTBIO, Biopanda	antigen, IgG/IgM/IgA
Melioidosis	InBios	antigen
Mononucleosis	Alere, Cortez Diagnostics	IgG/IgM
MRSA	Alere	antigen
Rubella	Standard Diagnostics, Biocan, Biogate Labs	IgG/IgM
S. aureus	Alere	antigen
Scrub Typhus	InBios, Cortez Diagnostics, Access Bio	IgG/IgM
Tetanus	Standard Diagnostics	IgG/IgM
Typhoid	Standard Diagnostics, Biocan, Biogate Labs, Mediven, Biopanda, Cortez Diagnostics	antigen, IgG/IgM
Zika	Biocan, Mediven	IgG/IgM
Sexually Transmitted Diseases		
Chlamydia	Standard Diagnostics, Cortez Diagnostics, Maternova	antigen
Neisseria Gonorrhoeae	Cortez Diagnostics, Maternova	antigen

pathogen	sources	Detection
Syphilis	Standard Diagnostics, Biocan, Alere, biolytical, Cortez Diagnostics, Biogate Labs, Maternova, Biopanda	IgG/IgM
Gastrointestinal		
Adeno	Standard Diagnostics, Biocan, Ameridx, Mediven	antigen
Cholera	Standard Diagnostics, Maternova	antigen
H. pylori	Standard Diagnostics, Biocan, Ameridx, Cortez Diagnostics, Biogate Labs, NTBIO, Biopanda	antigen, IgG/IgM
Rotavirus	Standard Diagnostics, Mediven	antigen
Fecal Antigens		
Calprotectin	Biocan	antigen
Clostridium difficile	Biocan	antigen
E. coli O157	Biocan	antigen
Giardia	Biocan, Alere, Ameridx	antigen
Lactoferrin	Biocan	antigen
Norovirus	Standard Diagnostics, Biocan	antigen
Procalcitonin	Biocan, Maternova, NTBIO	
Transferrin	Biocan	
Respiratory		
Influenza	Standard Diagnostics, Orasure, Biocan, Alere, Cortez Diagnostics, Mediven, Biopanda	antigen
Legionella	Standard Diagnostics, Alere, Cortez Diagnostics	antigen
Pneumonia	Alere, Biogate Labs	antigen, IgG/IgM
Respiratory Syncytial Virus (RSV)	Standard Diagnostics, Alere	antigen
Strep A	Standard Diagnostics, Alere, Cortez Diagnostics	antigen
Tuberculosis (TB)	Standard Diagnostics, Cortez Diagnostics, Biogate Labs	antigen
Cancer Markers		
Alpha-Fetoprotein (AFP)	Standard Diagnostics, Biocan, Cortez Diagnostics, NTBIO	antigen
Carcinoembryonic Antigen (CEA)	Biocan, NTBIO	antigen
Fecal Occult Blood	Standard Diagnostics, Biocan, Cortez Diagnostics, Mediven, NTBIO	antigen
Prostate Specific Antigen (PSA)	Standard Diagnostics, Biocan, Cortez Diagnostics, Biogate Labs, NTBIO	antigen
Cardiac Markers		
C-reactive protein	NTBIO	
Creatine Kinase Isoenzymes	Biocan, Cortez Diagnostics, Biogate Labs, NTBIO, Biopanda	antigen
Myoglobin	Standard Diagnostics, Biocan, Cortez Diagnostics, Biogate Labs, NTBIO, Biopanda	antigen
N-terminal B-type natriuretic peptide precursor (NT-proBNP)	Biopanda	antigen
Troponin I	Standard Diagnostics, Biocan, Cortez Diagnostics, Biogate Labs, NTBIO, Biopanda	antigen
Fertility Hormones		
human Follicular Stimulating Hormone	Cortez Diagnostics	direct

pathogen	sources	Detection
hCG	Standard Diagnostics, Biocan, Cortez Diagnostics, Mediven	direct
Luteinizing Hormone	Standard Diagnostics, Biocan	direct

Antigen indicates direct tests, IgG/IgM/IgA indicate serological tests.

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