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Development of Lateral Flow Assays using Gold Nanoparticles and Nanoshells

A compendium of recently published peer-review articles

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Lateral flow assays, also known as immunochromatographic assays, are self-contained, portable devices that meet the essential requirements for use as point-of-care diagnostics. They are low-cost, user-friendly and offer high sensitivity and selectivity for detection or quantification of bio-molecules in complex samples including blood, urine, saliva, and other fluids. The sensitivity of these assays depends in part on the properties of the nanoscale reporter particles that generate a signal during use. The reporter particles are labelled with a molecule, often an antibody or nucleic acid, that will recognize an analyte in the sample and bind to a specific location on the strip.

Gold nanoparticles are exceptionally strong absorbers of light and are therefore one of the most common types of reporter particles, producing ruby red test lines. Further, the gold surface has a natural affinity for proteins and can be easily functionalized with ligands for covalent coupling, facilitating the fabrication of nanoparticle-antibody conjugates.

Gold nanoshells with a 150 nm diameter produce a blue-green test line and provide a higher contrast per binding event. When incorporated into assays, they can increase sensitivity by 6–20 fold compared to traditional 40 nm gold nanospheres.

As highlighted below, gold nanoparticles and nanoshells developed by nanoComposix have been integrated into dozens of lateral flow assay systems, offering a clear advantage in stability, reproducibility, dynamic range, and sensitivity.

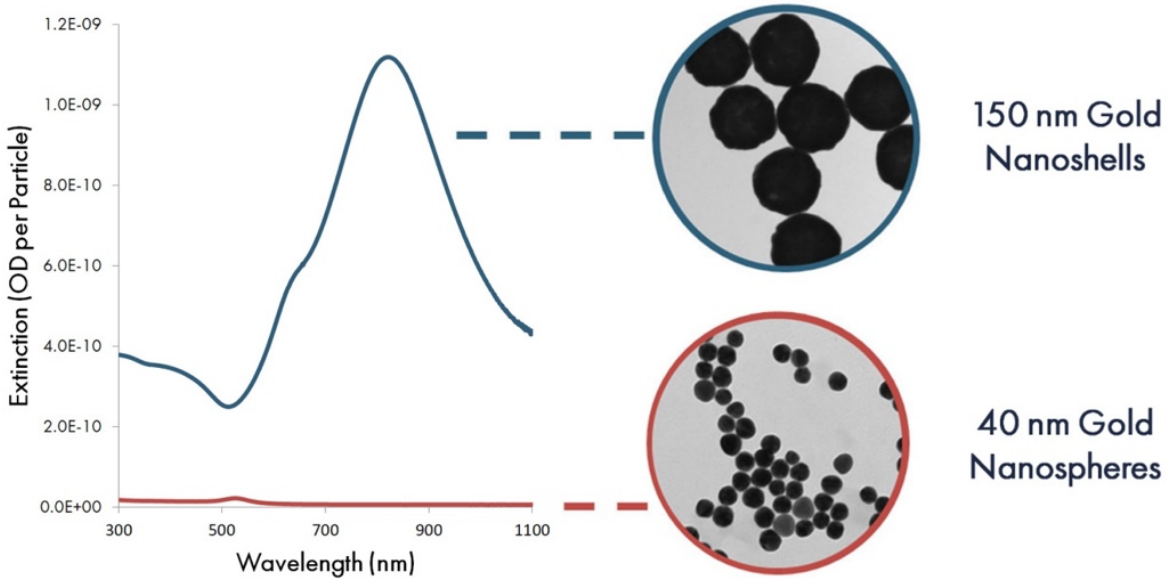

Figure 1. Per particle optical extinction (optical density a per particle) as a function of wavelength for 40 nm gold nanospheres and 150 gold nanoshells.

Infectious Diseases

The high prevalence of infectious diseases around world, combined with outbreaks driven by emerging pathogens such as Zika and SARS-CoV-2, is driving demand for new easy-to-use, affordable tests based on proven technology. Lateral flow assays offer a robust solution by delivering rapid, early, and accurate diagnosis of infectious diseases.

SARS-COV-2

Lateral flow assays have been developed to identify the presence of SARS-CoV-2 antigen, and thus an active infection, and to detect and characterize antibodies to SARS-CoV-2, providing important insights into the course of immunity and infection.

Frew, et al., describe development of a SARS-CoV-2 rapid antigen test for use in resource limited settings.[1] While not as sensitive as real-time reverse transcription polymerase chain reaction (RT-qPCR), rapid tests based on lateral flow technology are inexpensive, allow for patient testing in non-laboratory settings, and have a short turnaround time to results.

In this assay, mouse anti-nucleocapsid protein antibody is conjugated to 150 nm carboxyl gold nanoshells and chicken IgY was conjugated to 40 nm carboxyl gold nanoparticles as a control.[2] Preliminary performance data show that the assay met the acceptance criteria for sensitivity and specificity as outlined by the United States Food and Drug Administration (FDA) in emergency use authorization (EUA) guidance documents and is among the most sensitive lateral flow assays that do not require a reader.

Behrouzi and Lin sought to improve upon the sensitivity and specificity of point-of-care antigen tests that use optical detection schemes based on amplification of weak sensing signals to achieve low limits of detection (LOD).[3] The new diagnostic method relies on the localized surface plasmon resonance (LSPR) principle resulting from aggregation of antigen-coated 40 nm N-hydroxysuccinimide-dried gold nanoparticles (GNPs) to detect SARS-CoV-2 nucleocapsid proteins.

By conjugating GNPs with antibodies specific to the SARS-CoV-2 antigens, GNPs can aggregate in the presence of antigens. Accumulation of antigens around GNPs brings individual GNPs together, couples their plasmons, and changes the refractive index of the surrounding environment, causing a red-shift of the optical intensity spectrum. This results in the solution

turning blue, allowing a simple colorimetric method to detect presence of the virus by the naked eye. Assay results are observable by the naked eye in five minutes with an LOD of 150 ng/ml for nucleocapsid proteins.

Lake, et al., describes development of an assay that measures levels of neutralizing antibodies which block the SARS-CoV-2 viral receptor binding domain (RBD) from binding to cell surface angiotensin-converting enzyme 2 (ACE2).[4] This assay provides distinct advantages over RBD-ACE2-based competition ELISAs as it is a rapid, highly portable, semi-quantitative test, and is easily incorporated into clinical or research settings where traditional laboratory or neutralization tests are not practical.

The test leverages the interaction between RBD-conjugated gold nanoshells that bind ACE2 at the test line when RBD-neutralizing antibodies are absent or present at low levels in serum or whole blood. The authors conclude that this assay may prove useful in monitoring COVID-19 vaccine recipients as a correlate of protection. Since it requires only a drop of blood, individual use of the test might lead to more comprehensive longitudinal monitoring of protective humoral immunity and indicate when boosters might be required.

Tan, et al., developed a lateral flow assay to better understand how SARS-CoV-2 mutations mediate escape from the neutralizing activity of antibodies and identify anti-RBD antibodies for potential diagnostic and therapeutic uses. The assay includes anti-RBD and anti-nucleocapsid antibodies coated onto 40 nm citrate protected gold nanoparticles and was used to characterize seven anti-RBD monoclonal antibodies for binding activity, immunoassay pairing capability, and neutralizing activity toward variant SARS-CoV-2 RBDs. Based on the results of their studies, the authors conclude that the lateral flow assay offers an attractive and cost-effective alternative for characterizing antibody binding properties, epitope binning, and *in vitro* neutralizing kinetics of therapeutic antibodies and cocktails.

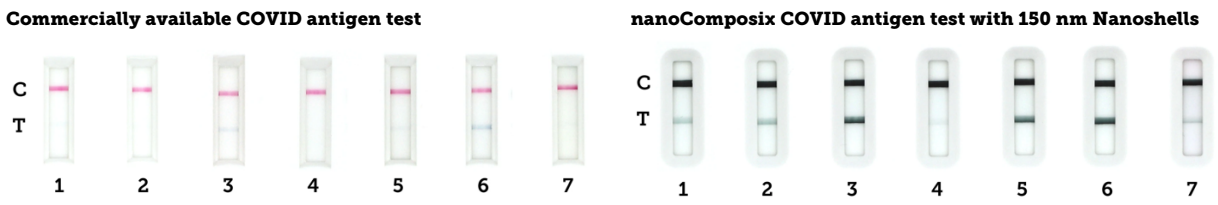

Figure 2. Comparison of test line intensity for 7 positive samples when run on a commercially available COVID antigen test using traditional colloidal gold particles versus a nanoComposix COVID antigen test using 150 nm nanoshells.

ONCHOCERCA VOLVULUS

Onchocerciasis, also known as river blindness, affects more than 15 million people, with 99% of cases found in sub-Saharan Africa. The disease is caused by the nematode *Onchocerca volvulus* and is transmitted by flies which inject infective larvae into human hosts. The World Health Organization (WHO) has sought to minimize the impact of onchocerciasis in Africa for nearly 30 years via mass drug administration (MDA) programs that deliver the anti-parasitic drug ivermectin to at-risk populations.

The program has recently shifted its focus from controlling to eliminating onchocerciasis. To enable this important step, new diagnostic tools able to detect minimal (0.1–1%) disease prevalence are needed. The ideal device is a rapid diagnostic test with high sensitivity and specificity, so that the number of false positives (<0.1%) would not overshadow an extremely low disease prevalence (<0.1%). While several tests to detect onchocerciasis already exist, none have the desired combination of sensitivity, specificity, and amenability for use in the field.

Gonzalez-Moa, et al., report development of a lateral flow assay using gold nanoshells, a novel type of plasmonic reporter nanoparticles as an alternative to 40 nm gold particles.[5] The gold nanoshells have a dark blue coloration and a per-particle extinction coefficient 35 times higher than that of 40 nm gold particles. Greater absorption translates into a 2–10 fold increase in the analytical sensitivity of the lateral flow assay employing gold nanoshells compared 40 nm gold particles. The authors report that the sensitivity, specificity, and cross-reactive values of the novel assay are comparable to those obtained by ELISA.

LOA LOA

While ivermectin MDA programs have achieved remarkable success towards elimination of onchocerciasis, these programs continue to be hindered in Central Africa by the occurrence of ivermectin-related neurological adverse events in a subset of individuals with high circulating levels of Loa loa microfilariae.

Pedram, et al., developed an inexpensive point-of-care lateral flow assay to identify people with a serological response to a specific validated marker of Loa loa infection. Availability of this test has the potential to make Loa loa prevalence maps more granular and help determine which communities can be safely included in MDA programs.

The rapid antibody test uses gold nanoshell particles, the optical properties of which can be modulated by varying the shell thickness. The nanoshells used in the assay are blue-to-black and have a per-particle extinction coefficient 35 times higher than that of red 40 nm gold commonly used in commercial lateral flow assays. As a result, the nanoshells appear darker to the human eye, even when 35 times more diluted, which translates into an increase in analytical sensitivity of 2–10 fold.

This assay is the first commercially available rapid antibody test for loiasis. Because of its sensitivity and specificity, the authors believe it can be adapted to accurately map the presence of this disease and facilitate programmatic decisions in MDA campaigns against river blindness in zones that are co-endemic with Loa loa.

Disease Biomarkers

Use of lateral flow assays as point-of-care devices for screening and monitoring various diseases and medical conditions is growing. These assays offer a more convenient, easy-to-use, low-cost format that can return results in minutes rather than hours or days as compared to more complicated tests, potentially improving patient outcomes.

ONCOLOGY

Srinivasan, et al., describe a proof-of-concept lateral flow test combined with a portable reader for quantification of total prostate-specific antigen (PSA) from a drop of serum within 20 minutes.[6]

The assay incorporates gold nanoshells as a label, allowing for a significant increase in the measured colorimetric signal intensity. This approach enables a five times lower detection limit when compared to 40 nm gold nanosphere labels, without a need for any additional signal amplification steps.

The authors believe this highly portable quantitative screening test for PSA has the potential to make prostate cancer screening more accessible where diagnostic labs and automated immunoassay systems are not available, reduce the turnaround time for results, and streamline both initial screening and post-treatment monitoring of patients.

A lateral flow assay has also been developed for monitoring recurrence of ovarian cancer.[7] Rates of relapse are high and current methods to detect recurrence are non-specific such as blood tests and ultrasound. A lateral flow assay was developed to quantitatively measure the ratio of human epididymis four (HE4) and creatinine (CRE) in urine, using 150 nm gold nanoshells. The authors report that the error in this assay is less than the difference required to detect recurrence and with further evolution, it could be used at home or in low-resource settings to provide timely detection of ovarian cancer recurrence and to monitor early responses to therapy. It may also prove beneficial in the early detection of ovarian cancer, for which there is no recommended screening test.

DOPAMINE

Disturbances in levels of the neurotransmitter dopamine play a significant role in many diseases including Alzheimer's, Parkinson's, and Huntington's, and the ability to monitor its level is important for early diagnosis and treatment monitoring. While current analytical methods are highly sensitive, they are relatively laborious and require sophisticated equipment and procedures, such as electrochemical detection, chemiluminescence, HPLC, colorimetry, and fluorescence.

Dalirirad and Steckl have developed a simple, low-cost lateral flow assay for measurement of dopamine levels in urine that has the potential to assist patients by providing in-home self-monitoring.[8] Dopamine duplex aptamers (hybridized sensor with capture probe) are conjugated to 40 nm gold nanoparticles. The detection method is based on dissociation of the duplex aptamer in the presence of dopamine, with the sensor part undergoing conformational changes and being released from the capture part. Hybridization between the complementary DNA in the

test line and the conjugated gold nanoparticle-capture DNA produces a red band, whose intensity is related to the dopamine concentration. Using this assay, the minimum detectable concentration was <10 ng/mL (65.2 nM), while the visual limit of detection was estimated to be ~50 ng/mL; the normal range of dopamine in urine is 52–480 ng/mL or 0.3–3.13 μM.

SALIVARY CORTISOL

The detection of stress levels using point-of-care devices to detect stress-related biomarkers such as cortisol is an area of growing importance for both healthy individuals experiencing stressful events and patients with a range of health issues. Cortisol increases in response to both physical and psychological stress, such as illness, injury, or depression; monitoring its level can serve as an important diagnostic indicator.

A disposable point-of-care aptamer-based lateral flow assay has been developed for the rapid detection of salivary cortisol.[9] The assay incorporates 40 nm gold nanoparticles and was designed based on the dissociation of a duplex cortisol–aptamer following introduction of the target cortisol in the clinically accepted concentration range. Increasing concentration of the target in the sample results in increased dissociation of the duplex aptamer and higher intensity at the test line of the assay. This simple and fast method offers high selectivity from closely related steroids and provides detection in the cortisol range of ~0.5–15 ng/mL, which is within the clinically accepted range for salivary cortisol. The LOD was 0.37 ng/mL, and the accuracy was confirmed by ELISA.

CEREBROSPINAL FLUID LEAK

Cerebrospinal fluid (CSF) leaks are a common and serious complication of many otolaryngology procedures and can also result from nonsurgical traumatic injuries such as facial and skull fractures. Currently, there are no rapid, noninvasive tests to rule out the presence of a leak. The gold standard laboratory-based test requires the sample to be sent for analysis which may require days to weeks.

To address this gap, Bradbury, et al., developed a lateral flow assay to quantify beta trace protein (βTP) in serum, an indicator of the presence of CSF leaks.[10] Detection of this protein was enabled by anti-βTP antibodies bound to 40 nm citrate capped gold nanoparticles. Validation studies demonstrated excellent predictive capabilities of the assay with the ability to distinguish between clinical specimens containing CSF and those without. The test for CSF leak detection is simple to

use, does not require any external equipment, and can be performed in approximately 20 minutes, making it well suited for use at the point-of-care.

Food-Borne Pathogens

Because of their simplicity, speed, and cost-effectiveness, lateral flow assays are also particularly suited for detection of disease-causing pathogens in food.

Feraudet Tarisse, et al., describe the development of highly sensitive, specific, and reliable lateral flow immunoassays for detection of classical and new staphylococcal enterotoxins.[11] These monoplex and quintuplex assays are based on single step immunochromatography using monoclonal antibodies directed at these different enterotoxins coupled to colloidal gold particles.

Detection limits in buffer were at least 300 pg/mL (11 pM) for all target toxins with no observed cross-reactivity. The authors believe the method could represent a reliable detection tool for staphylococcal enterotoxin types G, H and I, and be used for strain characterization, food safety, biological threat detection, and diagnosis of staphylococcal food poisoning.

A lateral flow assay incorporating polyclonal hen IgY antibodies has been developed to detect fumonisins in maize.[12] Fumonisins are among the most prevalent mycotoxins in maize, causing substantial economic losses and potential health risks in humans and animals. The intake of fumonisin-contaminated maize has been associated with oral, pharyngeal, and esophageal cancer in humans, as well as equine leukoencephalomalacia and porcine pulmonary edema. Due to its toxicity, international regulations restrict the maximum residue limit (MRL) of total fumonisins in raw maize to 4000 µg/kg.

High performance liquid chromatography (HPLC) or liquid chromatography tandem mass spectrometry (LC-MS/MS) have been used to detect fumonisins in maize but are laborious, time-consuming techniques that require specialized equipment. In contrast, lateral flow assays are cost-effective, easy to use, and suitable for on-site analysis. Several lateral flow assays for fumonisin detection in maize are available but have LODs ranging from 12–1200 µg/kg, which is a problem given the MRL of 4000 µg/kg. These assays generate an increased number of false-positive results that will be revealed by costly confirmatory methods.

In this novel assay, IgY was conjugated to 40 nm carboxyl gold nanoparticles via covalent immobilization and delivered an LOD equal to the MRL of 4000 µg/kg, enabling a reduction in the false positive rates observed with other lateral flow assays.

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[1] Frew E, Roberts D, Barry S, et al. A SARS-CoV-2 antigen rapid diagnostic test for resource limited settings. Sci Reports. 2021; 11:23009. https://doi.org/10.1038/s41598-021-02128-y.

[2] Tan E, Frew E, Cooper J, et al. Use of lateral flow immunoassay to characterize SARS-CoV-2 RBD-specific antibodies and their ability to react with the UK, SA and BR P.1 variant RBDs. Diagnostics (Basel). 2021; 11(7):1190. Published 2021 Jun 30. doi:10.3390/diagnostics11071190

[3] Behrouzi K and Lin L. Gold nanoparticle based plasmonic sensing for the detection of SARS-CoV-2 nucleocapsid proteins. Biosensors and Bioelectronics. 2022; 195:113669. https://doi.org/10.1016/j.bios.2021.113669

[4] Lake D, Roeder A, Kaleta E, et al. Development of a rapid point-of-care test that measures neutralizing antibodies to SARS-CoV-2. Journal of Clinical Virology. 2021; 145:105024. https://doi.org/10.1016/j.jcv.2021.105024

[5] Gonzalez-Moa M, Van Dorst B, Lagatie O, et al. A proof-of-concept rapid diagnostic test for onchocerciasis: exploring new peptide biomarkers and the use of gold nanoshells as novel reporter nanoparticles. ACS Infectious Diseases. 2018: 4, 6:912–917. https://doi.org/10.1021/acsinfecdis.8b00031

[6] Srinivasan B, Nanus D, Erickson D, et al. Highly portable quantitative screening test for prostate-specific antigen at point of care. Current Research in Biotechnology. 2021; 3:288-299. https://doi.org/10.1016/j.crbiot.2021.11.003

[7] Kight E, Hussain I, Bowden A, et al. Recurrence monitoring for ovarian cancer using a cell phone-integrated paper device to measure the ovarian cancer biomarker HE4/CRE ratio in urine. Scientific Reports. 2021; 11:21945. | https://doi.org/10.1038/s41598-021-01544-4

[8] Dalirirad S and Steckl J. Lateral flow assay using aptamer-based sensing for on-site detection of dopamine in urine. Analytical Biochemistry. 2020; 596:113637. https://doi.org/10.1016/j.ab.2020.113637

[9] Dalirirad S, Han D, Steckl J. Apatmer-based lateral flow biosensor for rapid detection of salivary cortisol. ACS Omega. 2020; 5 (51): 32890-32898. DOI: 10.1021/acsomega.0c03223

[10] Bradbury D, Kita A, Hirota K, et al. Rapid diagnostic test kit for point-of-care cerebrospinal fluid leak detection. SLAS Technology. 2020; 25(I)67-74. https://doi.org/10.1177/2472630319877377

[11] Féraudet Tarisse C, Goulard-Huet C, Nia Y, et al. Highly sensitive and specific detection of staphylococcal enterotoxins SEA, SEG, SEH, and SEI by immunoassay. Toxins. 2021; 13:130. https://doi.org/10.3390/toxins13020130

[12] Viet Tran T, Do B, Nguyen T, et al. Development of an IgY-based lateral flow immunoassay for detection of fumonisin B in maize. F1000 Research. 2019; 8:1042.