# Increased sensitivity of lateral flow assays using gold nanoshells

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## Introduction

The lateral flow immunoassay is a self-contained, portable diagnostic device that is easy to use, fast and inexpensive. The lateral flow test devices can be stored at ambient temperature, have a long shelf life, and provide diagnostic results without sample processing or additional equipment, making this format ideal for point-of-care and field-based diagnostics. Hundreds of millions of lateral flow assays are used each year to detect a wide variety of analytes.

**Figure 1.** Lateral flow assays indicate a positive signal based on the appearance of colored lines on a test strip.

The most common lateral flow assay configuration uses colloidal gold nanospheres to provide a readout, which appears as a red line when bound at a specific location on a membrane (Figure 1). There is also a positive control line to indicate test validity. The red color arises from the absorption of green and blue light by the gold nanoparticles. Because the optical properties of gold nanoparticles are dependent on size and shape, the sensitivity of lateral flow assays is limited by the intensity of the signal from the small (typically 40 nm in diameter) reporter particle. Sensitivity of analyte detection is critical. If the clinically relevant range of the analyte cannot be reached, the lateral flow strip will not have a commercial market.

NanoComposix has developed a gold nanoshell that typically results in 6 to 20 times greater sensitivity in lateral flow assays compared to 40 nm diameter gold colloid. NanoComposix's BioReady 150 nm gold nanoshell is a plasmonic reporter nanoparticle that consists of a 120 nm silica core coated with a thin (12-18 nm) shell of gold. The silica interior reduces the particle density relative to a solid gold nanoparticle, allowing for improved flow rates through a lateral flow membrane compared to a 150 nm solid gold sphere. Gold nanoshells have a large extinction cross section (*i.e*. an extremely intense color) with a peak extinction wavelength that is a function of the diameter of the core and the thickness of the shell. The peak extinction is 35-fold larger than 40 nm gold spherical nanoparticles (Figure 2). As the gold nanoshells have a higher per particle extinction cross section than 40 nm gold particles, fewer binding events at the test line are required to visualize results in a lateral flow test. Additionally, only minor modifications to existing protocols are required because the gold nanoshells have the same gold surface as spherical gold nanoparticles.

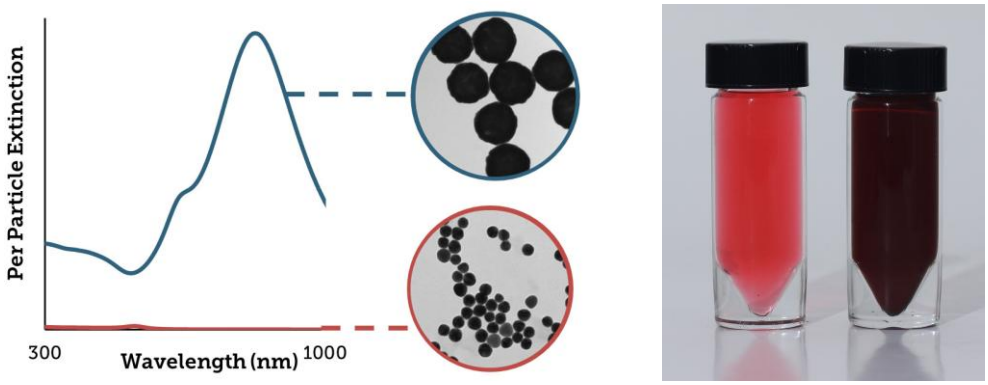


**Figure 2.** The graph shows150 nm BioReady gold nanoshells absorb light with a per particle extinction coefficient that is 35-fold larger than 40 nm gold spherical nanoparticles . The vial of 40 nm gold nanopartcles (A) and the vial of gold nanoshells (B) are at the same particle number concentration and highlight the difference in color intensity.

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Lateral flow assays that use colloidal gold nanospheres produce a red test line. The gold nanoshells produce a high contrast blue-grey line that is clearly visible to the eye. A 20 fold increase in sensitivity was obtained using gold nanoshells compared to 40 nm gold when detecting a human hormone (Figure 3). Similar sensitivity increases have been obtained in a wide variety of direct and competition assays including those for salivary cortisol, TSH, Troponin I, *b. burgdorferi*, onchocerceia, lymphatic filiariasis, malaria, Hepatitis B and many others.

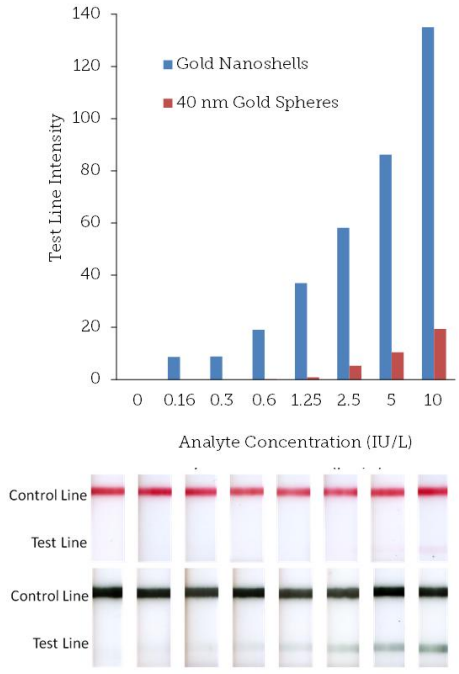


**Figure 3.** The BioReady 150 nm Gold Nanoshells (blue test line) are 20 times more sensitive in lateral flow assays than 40 nm gold spheres (red test line). *Produced in collaboration with ical-Q.*

**Nanoshell Fabrication:**

Nanoshells are manufactured using colloidal chemistry techniques at nanoComposix. Monodisperse, uniform 120 nm diameter silica cores are coated with a thin (12-18 nm) gold shell using a two-step process. First, small gold nanoparticles are bound to the silica surface. These small seed particles are grown larger until they coalesce into a complete shell (Figure 4). The final particles have a very narrow size distribution which is important for reproducibility in quantitative assays. The multi-seed method of shell growth leads to near elimination of crystallinity and faceting, which is typically an issue when growing larger gold nanoparticles.

Nanocomposix fabricates nanoshells in large lots (20 liters of 20 OD), which is sufficient for the production of hundreds of thousands of lateral flow tests. The nanoshells are functionalized with a carboxyl surface to allow for covalent binding to antibodies through EDC/Sulfo-NHS linkage chemistry. After functionalization, the nanoparticles are extensively washed into water (10X volume wash).

**Nanoshell Characterization:**

Each lot of nanoshells is extensively characterized to ensure high lot-to-lot consistency. Due to the plasmon resonance, a collective oscillation of the electrons in the gold shell, the gold nanoshells strongly interact with incident light. The “color” of the particle is dictated by the ratio of the size of the core and the thickness of the shell. This is monitored using UV-vis spectroscopy. The UV-vis spectrum is one of the primary characterizations of the gold nanoshells. Thinner shells on the same size core will have a longer peak extinction wavelength. Thicker shells will have a shorter wavelength peak. For lateral flow applications, the size of the core and the thickness of the shell have been optimized to maximize contrast in the visible and near-IR regions of the spectrum. Very high contrast lateral flow lines are observed in the visible and even higher signals are available in the infrared with the appropriate reader.

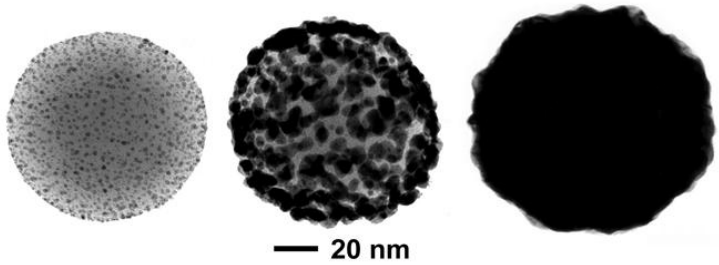


**Figure 4.** TEM images of gold shell fabrication . Initially, small gold nanoparticles are bound to the surface of a silica core (left). The gold nanoparticles are grown larger via an electroless deposition of a gold salt (middle). After sufficient gold has been reduced, the gold nanoshell is complete (right).

A comprehensive certificate of analysis is provided with each gold nanoshell lot (Figure 5). Transmission electron microscopy (TEM) is used to determine the

average and standard deviation of the nanoshell diameter. The particle concentration is calculated by TEM diameter and gold concentration is measured by ICP-MS. Based on the TEM determined shell thickness, a gold mass per particle can be calculated and the ICP-MS concentration is used to calculate particle concentration and surface area. Dynamic light scattering is used to measure the hydrodynamic particle size in solution and a hydrodynamic diameter that is within 20 nm of the TEM determined diameter verifies that the particles are not aggregated in solution. The mass concentration of the particles is determined with ICP-MS via the dissolution of the gold shell and measurement of the gold ion concentration in solution. The particle surface is functionalized with lipoic acid that tightly binds to the particle surface via a di-thiol bond and results in a carboxyl functionalized surface. Since the particles are suspended in a weak carbonate buffer, the pH varies between 5 and 8 but will immediately match the pH of any dilution buffers during use. The Zeta potential measures the surface charge of the particle and is strongly negative at neutral pH values due to the carboxyl surface. The extensive characterization of the physical and chemical properties of the nanoshells ensures that released nanoshell lots are highly reproducible.

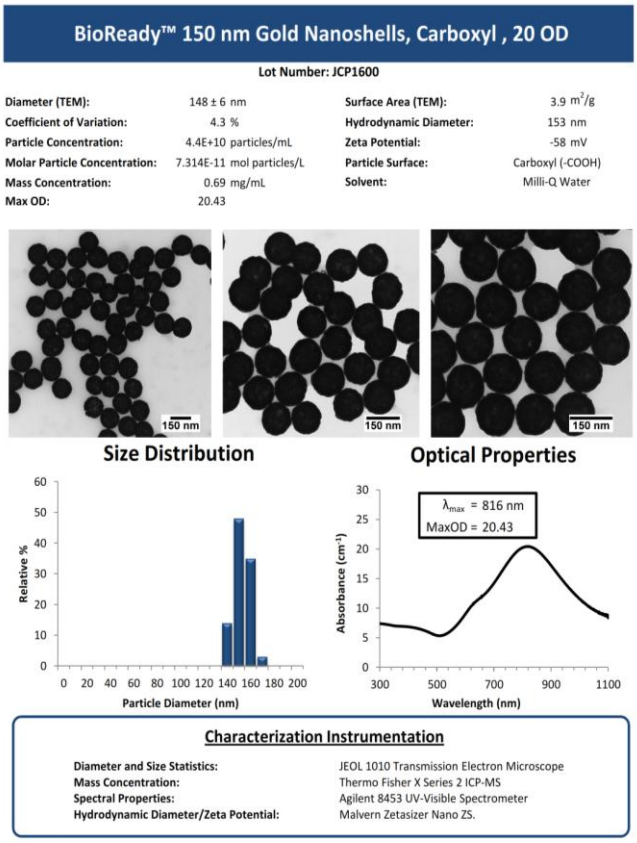


**Figure 5:** Representative certificate of analysis provided with every lot of material from nanoComposix.

**Nanoshell Conjugation:**

The nanoparticle/antibody conjugate is one of the most important components in a lateral flow assay. For gold nanoshells, covalent binding chemistry is used to link antibodies or other targeting agents to the particle surface. Purified antibodies that are suspended in a low ionic strength buffer are bound to the carboxyl surface using EDC/Sulfo-NHS chemistry. This forms a covalent amide bond between the antibody and the particle surface. Briefly, the carboxyl gold nanoshells are activated using EDC and sulfo-NHS reagents giving rise to amine reactive NHS ester termination. Immediately after activation, the particles are mixed with the antibody and allowed to incubate. The NHS esters then react with free amines from the antibody, forming amide bonds to the ligands on the gold surface. A quencher with an excess of free amines is subsequently added to deactivate any remaining NHS-esters and the particles are centrifuged and washed three times before resuspension in a conjugate diluent. Detailed antibody purification and conjugation protocols are available on our web site.

Covalent binding has a number of advantages over the traditional passive absorption techniques for preparing conjugates:

> ***Conjugate Stability:*** The conjugates are typically more robust and have increased stability in a wider variety of sample matrices and buffers. We have a number of examples where customers could not prepare a stable conjugate with passive absorption but were successful with covalent binding. Specific advantages include:

- Greater resistance to the extraction conditions for swab-based bacterial detection
- Better conjugate release and stability in a broad range of urine conditions
- Greater salt stability for phase separation
- Stable in buffers prepared with significantly higher volumes of detergents and salts compared to passively adsorbed conjugates
- Useful for conjugating antibodies such as IgM's which can be difficult using passive methods

***Antibody Ratio:*** By changing the proportions of antibody to particles and the reaction time, the number of antibodies per particle can be controlled. This is especially important for competitive assays where adjustment to the number of antibodies per particle can be used to bring the assay's dynamic range into the clinically relevant window. For direct sandwich assays, control over antibody number is also important. With a high affinity antibody that has rapid binding kinetics, a lower number of antibodies per particle is optimal since even one antibody with a bound antigen has a high probability of binding to the test line. However, if the antibody kinetics are slow or if the antibody binds with less affinity, more antibodies per particle are required to ensure that there is a binding event at the test line before the nanoparticle conjugate is wicked past the test line. For low analyte concentrations that require maximum assay sensitivity, it is important to have as many nanoparticle conjugates as possible with a bound antigen that can bind at a test line. For example, if only 100 analytes are available in a sample and each nanoparticle conjugate had a single antibody, 100 particles could potentially bind at the test line. However, if each conjugate has many antibodies, multiple analytes could bind to each particle reducing the total potential number of nanoparticles that can bind at the test line. For each assay, there is an optimal antibody loading ratio which needs to be determined empirically.

- In our hands, covalent binding is almost always better in competitive assays compared to physisorption.
- Better results when detecting antibodies as the analyte
- Better results when using streptavidin/biotin based assays

***Reduced Development Time:*** One of the most important advantages of covalent binding chemistry is that it is much quicker to produce a functional conjugate with covalent binding compared to physisorption since salt and pH conditions do not have to be swept to get a functional conjugate. This greatly decreases the time it takes to screen the dozens of antibody pairs necessary to identify a combination that will be used for further assay development.

**Nanoshell Cost Per Strip**

Historically, producing conjugates with covalent binding has been expensive, limiting their use for low margin immunoassays. At nanoComposix our scaled manufacturing process has allowed us to provide carboxyl functionalized nanoshells at just $.75 an OD-mL (equivalent to $750 per L of 1 OD, or $15 per mL of 20 OD), less than what many vendors charge for standard gold nanoparticles. However, the cost per OD is not the only parameter in determining the per strip cost. The gold nanoshells are larger and have a higher OD per particle. Often, our customers require 3-5 times more nanoshells on an OD basis when compared to 40 nm gold particles in the final optimized assay in order to maximize sensitivity. While this does increase the cost per strip compared to spherical gold, in most cases, the assay sensitivity improvement justifies the additional costs associated with requiring a higher OD per strip. Further cost savings can be achieved when purchasing at larger volumes.

**Lot Reproducibility and Commercial Supply**

For commercial production it is essential that there is low lot-to-lot variability so that assays do not need to be modified during manufacture. This is especially important for semi-quantitative or quantitative assays that are providing more than a "yes/no" answer. Batch to batch reproducibility studies have been performed with 3 lots of nanoshells both internally and by our customers. Typical lot-to-lot variable numbers are shown in Figure 6.

Upon request, lot sizes up to 400,000 OD-mL can be produced which reduces the effect of small variances in different lots. All lots are manufactured with SOPs and batch records compliant with ISO 13485 standards. Quality audits at our site are available on request.

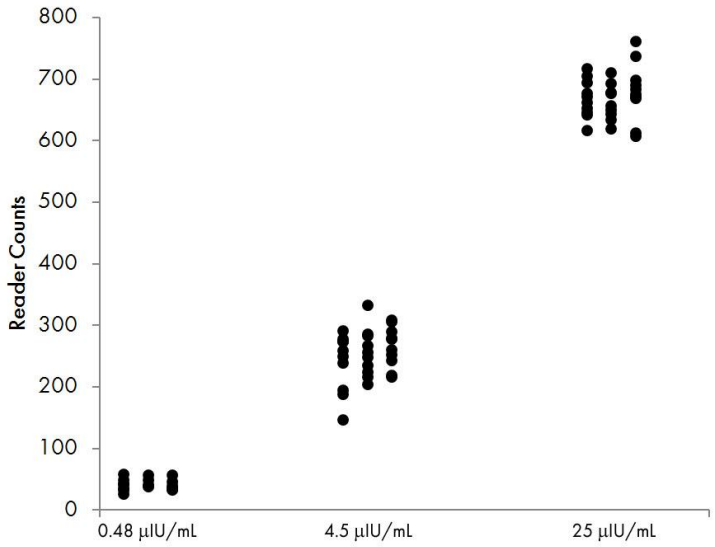


**Figure 6.** Three lot inter-batch and intra-batch variability for a lateral flow assay with 0.48, 4.5 and 25 μIU/mL concentrations of an analyte in a sandwich assay using gold nanoshells as the probe and read with a Qiagen ESEQuant benchtop reader.

**Case Studies**

Gold nanoshells have been integrated into dozens of lateral flow assay systems. In all cases, there has been a clear advantage in stability, reproducibility, dynamic range, or sensitivity. A number of case studies that describe the advantages of gold nanoshells are provided below:

*Hormone Detection:* Nanoshells were utilized in a hormone detection assay where 150 nm gold nanoshells achieved an 8X lower limit of detection compared to 40 nm gold which was necessary in order to measure the clinically relevant range. A 510k application with the data is under review by the FDA.

*Salivary Cortisol:* A salivary cortisol assay was developed both with 40 nm gold nanospheres and 150 nm diameter nanoshells. This is a semi-quantitative competition assay where a large dynamic range is required to track diurnal baselines and cortisol spikes. Sensitivity is not an issue as both the 40 nm nanospheres and the nanoshells could detect the lowest clinically relevant range of 0.5 ng/mL. However, with gold nanoshells, the dynamic range was increased by a factor of 10.

*Troponin I:* A 10X increase in sensitivity was obtained when measuring Troponin I analyte in serum using gold nanoshells compared to 40 nm gold.

*Malaria:* An 8X increase in sensitivity was obtained in a Malaria test developed by a partner.

**Conclusions:**

By retaining all of the advantages of traditional gold nanosphere probes with the additional benefit of dramatically increasing the contrast per binding event, gold nanoshells provide a simple and straightforward method for increasing lateral flow sensitivity. This increase in sensitivity has enabled new tests that can now access clinically relevant concentrations of analytes, have increased dynamic range or, by trading speed for sensitivity, can reduce the time to readout. Covalent coupling to the particle surface reduces assay development time and increases the conjugate stability in a variety of sample matrices. When coupled with inexpensive mobile readers to provide quantitative analysis, gold nanoshells have the potential to enable a new generation of commercial lateral flow assays.