Enhancing Sensitivity in Lateral Flow Assays using Passively Conjugated Gold Nanoshells

Lateral flow assays (LFAs) are simple to use, disposable diagnostic devices that can test for a wide variety of biomarkers in diverse sample types, including saliva, blood, urine, and food. Standard lateral flow tests generate an optical signal that arises from strongly colored reporter probes, often nanoparticles, bound to test lines on a white nitrocellulose strip. Given the critical role of the probes, their selection is one of the most important decisions made during the planning of a new lateral flow assay. The reporter particle choice impacts not only the achievable sensitivity, stability in the sample matrix, cost of the assay, and development time, but also determines whether a reader is required for final signal readout. To maximize sensitivity for LFA, nanoComposix developed blue-green 150 nm Gold Nanoshells (GNS), first provided as a carboxyl functionalized particle to enable covalent conjugation via EDC/Sulfo-NHS coupling chemistry, and now available in a citrate capped surface for physisorption, commonly known as passive conjugation.

Passive Conjugation to Gold Nanoparticles

Robust and effective binding of an antibody to the surface of a reporter particle is critical for obtaining the target sensitivity and selectivity of a lateral flow assay. A simple and effective method of creating nanoparticle conjugates is to simply mix citrate-capped gold nanoparticles with an antibody. The antibody binds to the surface of the particle by taking advantage of intermolecular forces between molecules and surfaces (e.g. van der Waals and ionic forces) and can target biomarkers and analytes with high sensitivity and specificity. New BioReady™ 150 nm Citrate Gold Nanoshells are suspended in a weak (0.2 mM) sodium citrate buffer to enable passive conjugation techniques by stabilizing the nanoshells with weakly associated citrate molecules that can be readily displaced by antibodies and other proteins under the correct buffer and pH conditions.

Optical Response of Nanoshells

One of the most common types of particles used in diagnostic assays are gold nanoparticles, often referred to as gold colloid. Gold nanoparticles have unusual optical properties that make them exceptionally strong absorbers of light and traditional 40 nm diameter gold nanospheres have a peak absorbance at ~520 nm, resulting in a ruby red colored test line in lateral flow assays. By comparison, gold nanoshells with a 150 nm diameter provide a blue/green test line and a higher contrast per binding event than 40 nm gold nanospheres. **Figure 1** below shows a comparison between the optical extinction (scattering + absorption) per particle of 40 nm gold nanospheres and 150 nm gold nanoshells.

Due to their stronger extinction per particle, each nanoshell binding event has a much higher contrast against the nitrocellulose substrate, which can deliver increased assay sensitivity. An image of test and control lines using gold nanoshell reporters with a corresponding scanning electron microscope image is shown in **Figure 2**. Individual nanoshells bound to antibodies immobilized on the nitrocellulose membrane can be identified as light-colored spheres on the darker nitrocellulose fibers. Even at the relatively sparse coverage density on the membrane, the control line appears very dark demonstrating that each nanoshell binding event generates a high contrast signal against the white nitrocellulose background.

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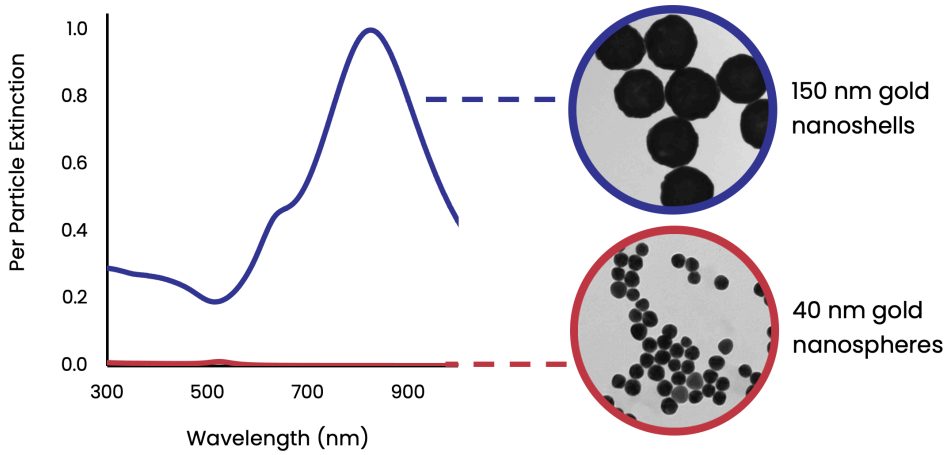

Figure 1: Extinction per particle of traditional 40 nm gold nanospheres versus 150 nm gold nanoshells.

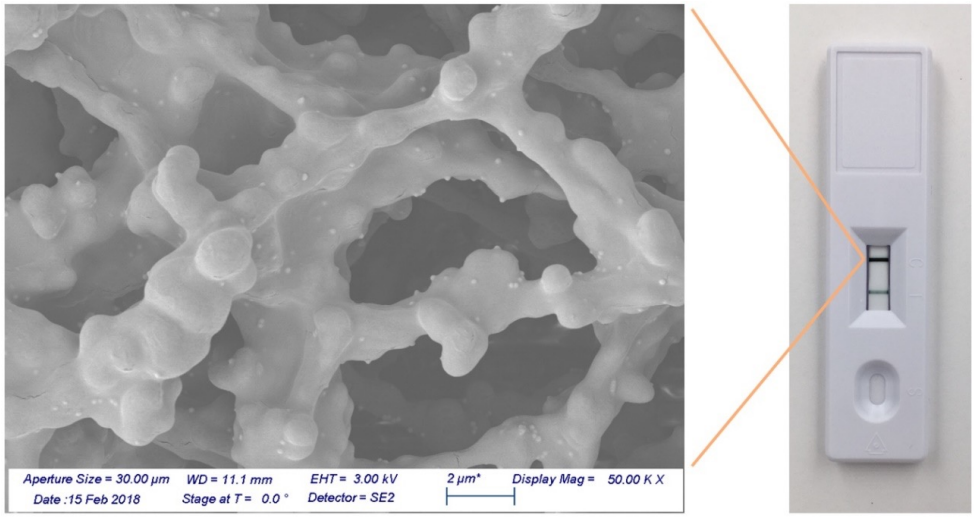

Figure 2: Gold nanoshells bound to a nitrocellulose membrane..

Performance Comparison in Thyroid Stimulating Hormone (TSH) and SARS-CoV-2 Nucleocapsid Protein (SNP) Lateral Flow Assays

The unique optical properties of nanoshells enable them to offer enhanced sensitivity in lateral flow assays, regardless of whether covalent or passive conjugation techniques are employed to produce the reporter probe conjugates. Previous work in **Performance Comparison of Commonly Used Nanoparticle Probes in SARS-CoV-2 Nucleocapsid Protein LFA** compared the performance of covalently conjugated BioReady™ 150 nm Carboxyl Gold Nanoshells against other covalently conjugated probes while this investigation compared passive conjugates prepared using BioReady™ 150 nm Citrate Gold Nanoshells and 40 nm Citrate Gold Nanospheres in two assays, Thyroid Stimulating Hormone (TSH) and SARS-Cov-2 Nucleocapsid Protein (SNP).

The conjugates for each particle type and assay were optimized one at a time and special considerations were made for each particle type including sweeps for optimal pH, reaction buffer, antibody loading, block buffer, and blocking duration and temperature. Strips were prepared, testing

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TSH concentrations between 0–1 ng/mL and SNP concentrations between 0–50 ng/mL, with 8 µL of conjugate at 10 OD used per strip. The strips were then allowed to run for 20 minutes before being read on a Lumos Leelu Reader with the appropriate illumination.

Once the signal intensities and standard deviations for each probe and sample level were calculated, the following formulas were used to determine the Limit of Black (LoB), and subsequently, the Limit of Detection (LoD) for each conjugate using the formulas below[1].

LoB = mean of blank (i.e., 0 ng/mL) + (1.645 × standard deviation of blank)
LoD = LoB + (1.645 × standard deviation of low concentration sample)

As shown in **Table 1** below, the Gold Nanoshells achieved a lower calculated LoD in both assays.

Signal intensities from the Leelu Reader for each analyte concentration sweep are shown below in **Figure 3** and **Figure 4**. The nanoshells demonstrated higher sensitivity and lower Non-Specific Binding (NSB) compared to the 40 nm gold nanospheres in both assays.

	Gold Nanoshells	Gold Nanospheres
LoD SNP Assay (calculated)	3 ng/mL	4 ng/mL
LoD TSH Assay (calculated)	0.1 ng/mL	0.3 ng/mL

Table 1: The calculated Limits of Detection (LoD) for each particle type in both assays investigated.

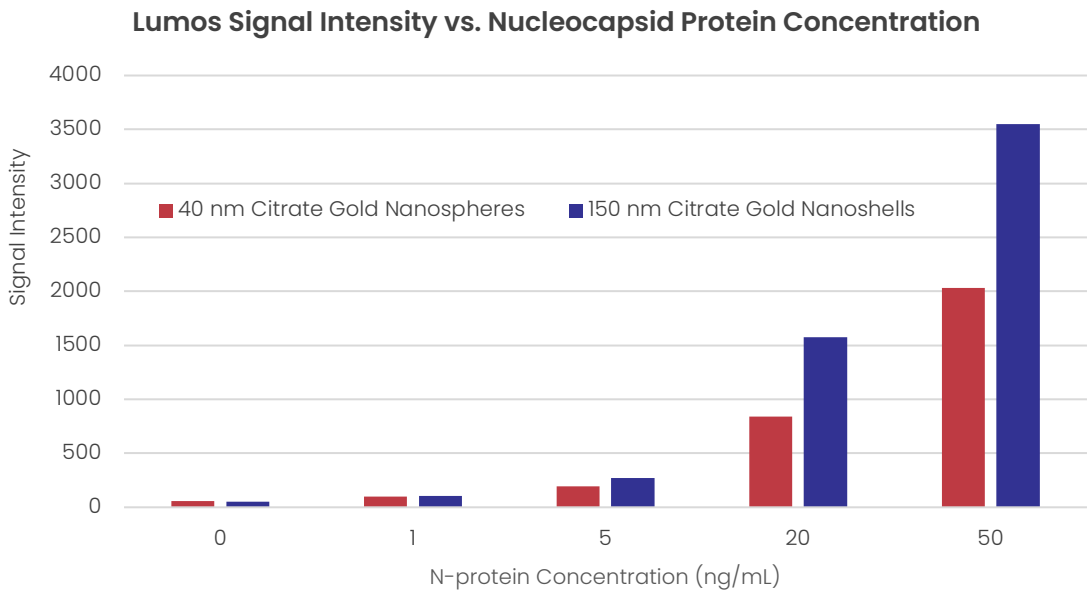

Figure 3: Reader signal intensities collected from conjugates made with 40 nm Citrate Gold Nanospheres versus BioReady™ 150 nm Citrate Gold Nanoshells in the SARS-Cov-2 Nucleocapsid Protein assay.

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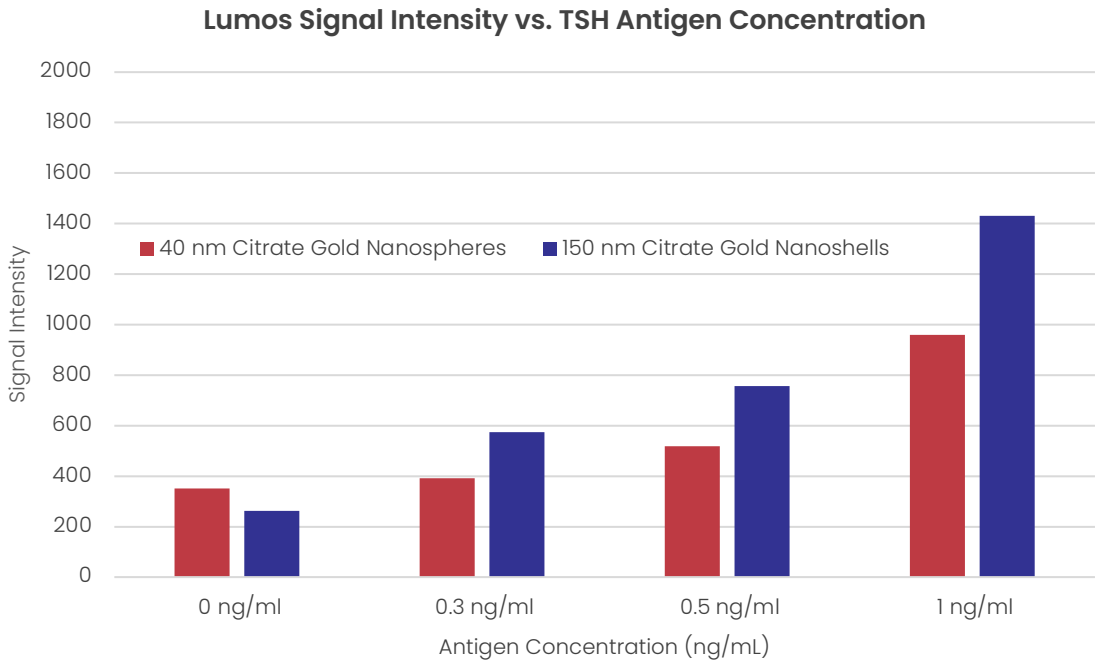

Figure 4: Reader signal intensities collected from conjugates made with 40 nm Citrate Gold Nanospheres versus BioReady™ 150 nm Citrate Gold Nanoshells in the Thyroid Stimulating Hormone assay.

In addition to the improved sensitivity offered by nanoshells in these two assays, the nanoshells demonstrated a much lower optimal antibody loading than 40 nm gold nanospheres, which translated to less antibody required per strip. Although the cost per particle of nanoshells is typically higher than 40 nm gold nanospheres, **Table 2** shows that the cost per strip of particles and antibody together is reduced by over 20% for the SNP assay when nanoshells are used.

	Gold Nanoshells	Gold Nanospheres
Probe Color on Strip	Green/Blue	Red
Probe Size	150 nm	40 nm
Capping Agent	Citrate	Citrate
Antibody Loading	30 µg / 1 mL at 20 OD	60 µg / 1 mL at 20 OD
Volume of Conj. per Strip	8 µL at 10 OD	8 µL at 10 OD
Amount of Ab per strip (µg)	0.12	0.24
Cost per strip (Ab + probe)*	$0.11	$0.14

Table 2: Summary of particle properties, per-strip antibody usage, and antibody plus particle cost per strips for the SARS-Cov-2 Nucleocapsid Protein assay assuming purchase of 20 OD BioReady™ particles at 1 L scale.

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Conclusion

Passive conjugation offers an easy and effective method to biofunctionalize gold nanoparticles, and in the two assays explored, passive conjugates prepared using BioReady™ 150 nm Citrate Gold Nanoshells offered improved sensitivity and reduced antibody usage relative to traditional 40 nm gold colloid. With both performance and cost benefits, the nanoshells offer a significant advantage over 40 nm gold nanospheres.

Contact us today to receive a quote or learn more about using BioReady™ Gold Nanoshells for your assay.

For more information, visit fortislife.com

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References

1. Forootan, Amin et al. “Methods to determine limit of detection and limit of quantification in quantitative real-time PCR (qPCR).” Biomolecular detection and quantification vol. 12 1-6. 29 Apr. 2017, doi:10.1016/j.bdq.2017.04.001

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