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Performance Comparison of Commonly Used Nanoparticle Probes in SARS-CoV-2 Nucleocapsid Protein LFA

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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). In this study, these nanoshells, along with 40 nm Carboxyl Gold Nanospheres (CGN), Europium latex, and colored latex probes, were conjugated to an anti-SARS CoV-2 nucleocapsid antibody and incorporated into a lateral flow sandwich assay for comparison. Performance of the conjugates was evaluated by calculating the Limit of Blank (LoB), Limit of Detection (LoD), and overall signal intensity as a function of antigen concentration for each of the probes. In addition to this quantitative LoD comparison, considerations about conjugate preparation such as antibody loading, optimal probe concentration for conjugation, handling, and stability were also evaluated.

Introduction

Depending on the assay in development and the desired outcome of the work, the process of lateral flow assay optimization can require several months or longer. The scope of work outlined in this white paper was confined to approximately 4 weeks, performing the minimum number of experiments needed to eliminate any observable aggregation, colloidal instability, non-specific binding (NSB) of the conjugate, and to obtain reasonable signal intensities and background clearing by adjusting conjugate loading.

Once optimized conjugation conditions were identified, all conjugates were tested in a single, large testing event using the same antigen, strips, and reader (with Europium requiring a fluorescent reader) to minimize variability. Data collected were analyzed to estimate the LoB, LoD, and overall signal intensity as a function of antigen concentration for each of the probes.

Materials and Method

Commonly used nanoparticle probes, including 300 nm colored latex and Europium latex were acquired from external vendors, while 40 nm Carboxyl Gold Nanospheres and 150 nm Gold Nanoshells were prepared in-house by nanoComposix. All particles were available with a carboxylated surface to provide a means for covalent antibody conjugation using EDC/NHS coupling chemistry.

When available, the manufacturer-provided protocols were used as a starting point for conjugation with subsequent optimization to identify the most suitable conjugation conditions for the protein used in this study.

The conjugates were optimized one at a time and special considerations were made for each particle type. Optimizations for conjugates included:

- Reaction (coupling) buffer to identify best conditions under which to perform antibody binding while maintaining colloidal stability of the particles
- Block buffer to determine best way to block particles and reduce non-specific binding/aggregation observed on the strip
- Conjugate loading titration (i.e., volume and concentration) to ensure a strong Test Line and Control Line are observed while maintaining a clear background on the strip
- Centrifugation conditions to ensure that yield was maintained, and residuals were removed

Triplicate strips were prepared for testing nucleocapsid concentrations between 0–500 ng/mL. Strips were run using 8 µL of conjugate at various concentrations, 20 µL of antigen-containing sample, and 30 µL of running buffer. The strips were then allowed to run for 20 minutes before being read on a Lumos Leelu Reader with the appropriate illumination. Green illumination was used for the red colored particles, 40 nm Carboxyl Gold Nanospheres and colored latex, while red illumination was used for the Gold Nanoshells. To measure the fluorescence intensity of the Europium, a separate reader, emitting at 625 nm, was used.

Results

Signal intensities collected from the Leelu Reader(s) are provided in **Figure 1**.

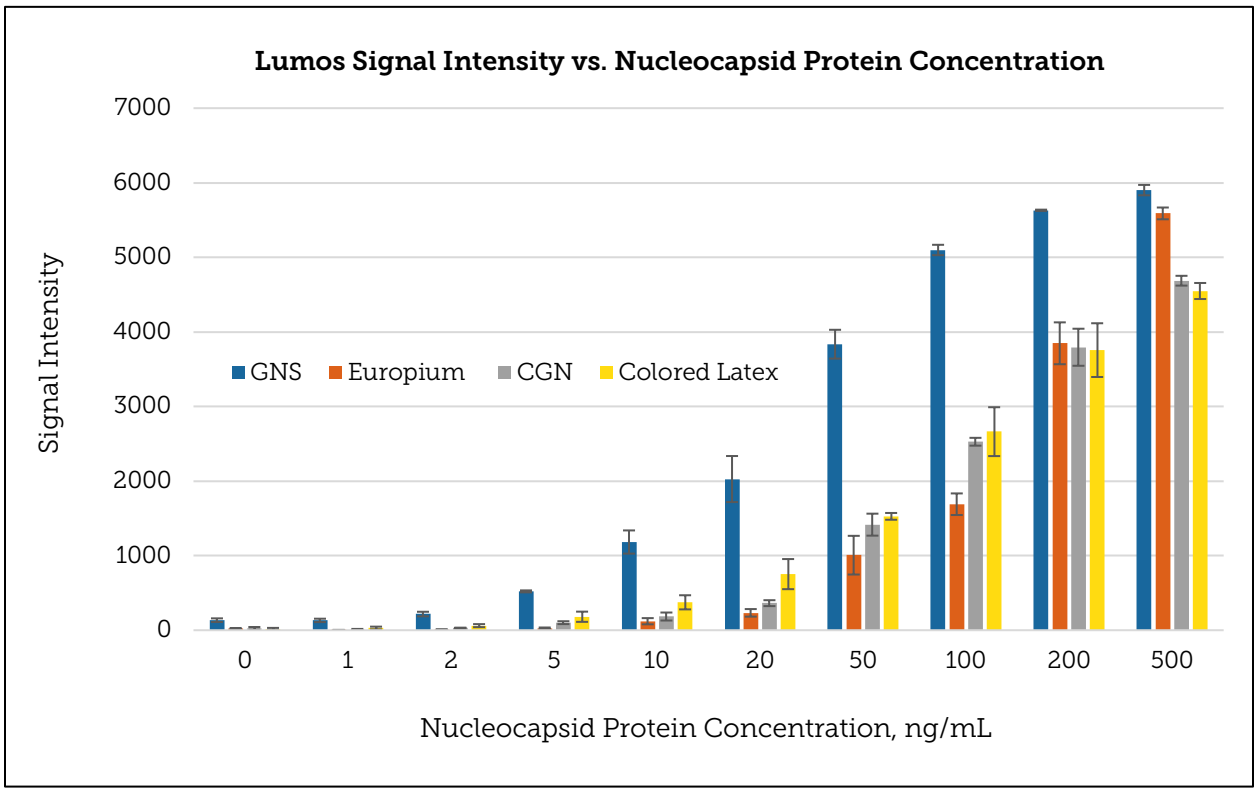


Figure 1. Reader signal intensities collected from conjugates made with various nanoparticle probes.

Once the signal intensities and standard deviations for each probe and sample level were calculated, the following formulas were used to determine the LoB and LoD for each conjugate using the formulas below.

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)

Calculation reference: ncbi.nlm.nih.gov/pmc/articles/PMC5496743/

	Europium	*GNS*	*Colored latex*	*CGN*
LoD (calculated)	11 ng/mL	2 ng/mL	2 ng/mL	6 ng/mL

Table 1. Calculated LoDs for each of the conjugates measured.

As shown in **Table 1**, both colored latex and Gold Nanoshells had the same calculated LoD. **Figure 2** compares strips run at nucleocapsid protein concentrations of 10 ng/mL with GNS and colored latex.

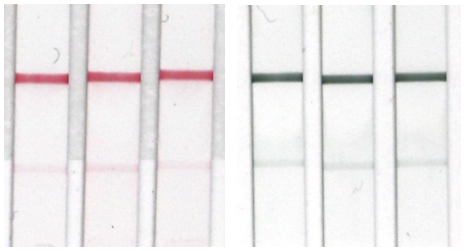
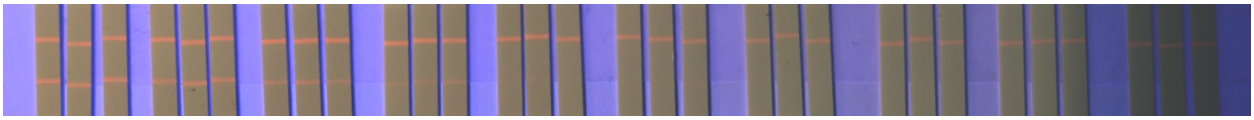


Figure 2. Colored latex (left) and Gold Nanoshells (right) at 10 ng/mL NP concentration.

In addition to the improved visual signal when using the nanoshells, the amount of antibody required to produce 1 mL of final latex conjugate is 3 times that needed to produce 1 mL of equivalent GNS conjugate. This emphasizes, that although latex has a lower cost per particle, the per strip cost of latex conjugate is 50% higher than GNS due to the increased amount of antibody required. The impact of increased antibody usage per strip is especially important when working with antibodies that are difficult to produce or source. Below, **Table 2** summarizes antibody loadings and probe specifications, while **Figures 3, 4, 5, and 6** show the triplicate strip runs for each probe type investigated.

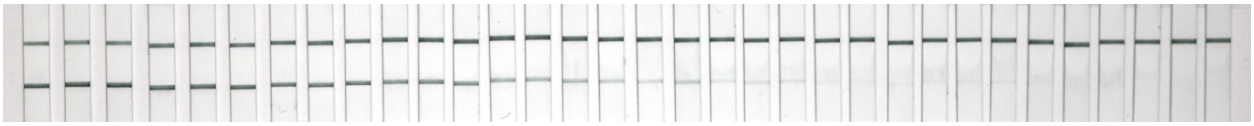
	Europium	*GNS*	*Colored latex*	*CGN*
Probe Color	Fluorescent	Green/Blue	Red (alternate colors available)	Red
Probe Size	300 nm	150 nm	300 nm	40 nm
Antibody Loading	50 µg / 1 mg	30 µg / 1 mL at 20 OD	50 µg / 1 mg	60 µg / 1 mL at 20 OD
Final Conj. Volume & Concentration	1 mL at 0.1 % (1 mg/mL)	1 mL at 20 OD	0.5 mL at 0.2% (2 mg/mL)	2 mL at 10 OD
Volume of Conj. per Strip	8 µL	8 µL	8 µL	8 µL
Amount of Ab per strip (ug)	0.40	0.24	0.80	0.24
Cost per strip (Ab + probe) relative to GNS	–15%	0%	+50%	–30%
LoD (calculated)	11 ng/mL	2 ng/mL	2 ng/mL	6 ng/mL

Table 2. Comparison of relevant data of all nanoparticle probes and conjugation conditions.



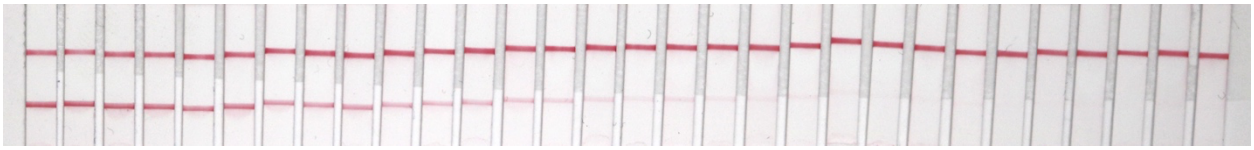
500 ng/mL *0 ng/mL*

Figure 3. Strips run with Europium conjugate using a small UV lamp to illuminate the strips for visualization. The signal intensity observed in this image may not accurately reflect quantitative data collected.



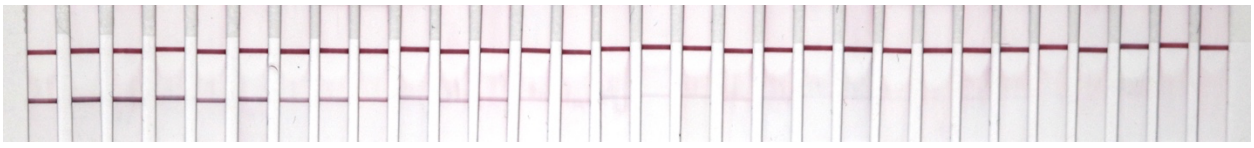
500 ng/mL *0 ng/mL*

Figure 4. Strips run with Gold Nanoshells conjugate.



500 ng/mL *0 ng/mL*

Figure 5. Strips run with colored latex conjugate.



500 ng/mL *0 ng/mL*

Figure 6. Strips run with 40nm Carboxyl Gold Nanospheres.

Conclusion

Both colored latex and GNS had the highest level of sensitivity, making these two probes the most suitable options for assay development where a low LoD is essential to the success of the assay. Other considerations such as cost, ease of use, reader type, and processing time are also important when selecting a probe for an assay. Below is a summary of these considerations for each probe type investigated in this study. The GNS demonstrated both exceptional performance and a low cost per strip due to the low antibody loading required.

EUROPIUM

Pros	Cons
• Robust during centrifugation and exposure to high salt concentration buffers, etc. • Clean background on strips • Competitive cost per strip (Ab + Particle)	• Increased overhead cost and complexity due to fluorescent reader requirement. • More challenging to troubleshoot conjugate or flow issues due to inability to visualize the conjugate as it migrates up the strips • Susceptible to photobleaching if not properly handled

GOLD NANOSHELLS

Pros	Cons
• Highest sensitivity • Strongest signal across multiple sample concentrations • Low Ab loading required, 0.24 ug per strip • Dark color on strip provides quick and easy visual interpretation	• More sensitive to processing conditions such as salt concentration and centrifugation • Limited number of suppliers • Higher background signal compared to other conjugates

40 NM CARBOXYL GOLD NANOSPHERES

Pros

- Low Ab loading required, 0.24 ug per strip
- Available from multiple vendors
- Lowest cost per strip (Ab + Particle)

Cons

- Weaker signal when compared to the other probes in this study

COLORED LATEX

Pros

- High sensitivity
- Runs with a clear background
- Robust during centrifugation and exposure to high salt concentration buffers

Cons

- High Ab loading required
- Contains free dye that can lead to signal variability, if not properly removed