White Paper

Optimization & Comparison of Four Different Gold Colloid Products for use in Lateral Flow

Evaluation of Multiple Gold Colloid Products for Use in a Lateral Flow Applications

Introduction

Gold colloid is a popular choice when choosing a nanoparticle for use in lateral flow assays. The use of gold colloid for passive conjugations has many advantages such as having a simple, quick protocol along with being relatively inexpensive. However, there are many gold colloid products available on the market for passive antibody conjugations and it can be challenging to choose a gold colloid product and supplier for your assay. This study explores the use of four 40 nanometre (nm) gold colloid products from four different suppliers. The gold colloids were conjugated using the protocol provided by the supplier with the aim of assessing the optimization protocols and the resulting gold colloid conjugate.

Gold colloid conjugation is a passive process depending mainly on ionic, hydrophobic, and dative binding. Hence, gold colloid is highly sensitive to the environment it is in during conjugation. The optimization of gold colloid conjugations typically involves modifying the pH and detector antibody concentration, followed by measurement using three main techniques – salt testing, ultraviolet visible spectrophotometry (UV-VIS) and lateral flow testing (Hermanson, 2013).

Salt testing with sodium chloride (NaCl) involves the addition of salt to the gold colloids. This technique is used because gold colloids are negatively charged and can repel themselves, causing the gold colloid particles to remain monodisperse in solution. However, when you add salt at high concentrations the negative charges on the gold colloid can become “masked” or altered. The gold colloids may then, as a result, aggregate out of solution. In an optimal conjugation environment , the gold colloids should be stable, and no aggregation will be seen. In a non-optimal environment, the salt will cause the gold colloid to aggregate which results in a visible colour change from red to purple (Hermanson, 2013, Momeni, 2022).

UV-VIS can also be used to test the optimal conjugation conditions. Gold colloid absorbs light at specific wavelengths. Hence, gold colloids have a very characteristic absorbance spectrum. When gold colloids are unstable, their absorption spectrum changes in two ways - the spectrum’s curve becomes broader and the spectrum has a higher absorption at longer wavelengths. Hence, the most stable spectrum will have the narrowest curve and lowest absorption at longer wavelengths (~580-650 nm) (Hermanson, 2013, Shengqiang, 2020, Haiss, 2007).

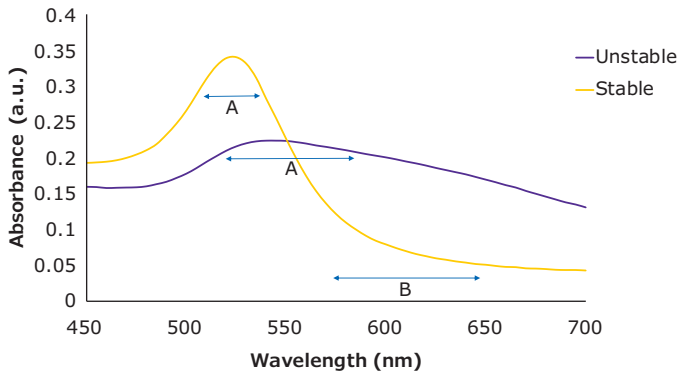


Figure 1 – Figure showing the changes in the absorbance spectrum when gold colloid particles are stable or unstable. “A” indicates the width of the curve changing from narrow (stable) to wide (unstable). “B” shows the differences in absorption at longer wavelengths. a.u. represents arbitrary units, nm represents nanometre.

Lateral flow testing involves testing a fully assembled lateral flow strip with the desired conjugate. The strips are then run using the appropriate analyte and matrix. This step is important because it will test for sensitivity, specificity, and aggregation on the strips.

This study involves the investigation of each of these techniques with four gold products. Each gold colloid supplier has provided a protocol for optimising the pH and detector antibody concentration using UV-VIS and/or salt. In addition, a Hepatitis B surface antigen (HBsAg) assay was used as the model lateral flow test system for all four gold colloid products.

Results

The four products tested are labelled A-D. For simplicity and clarity on the optimization process, only product A will be shown in the images below. Product B-D are discussed.

pH Testing

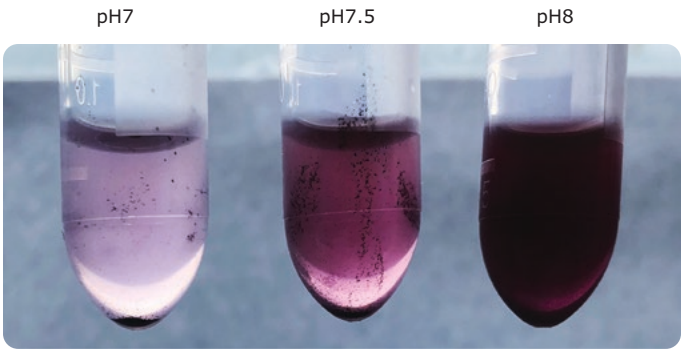


Figure 2 – Image of the gold colloid conjugates after the addition of salt with product A in the pH range 7-8.

Product A showed an increase in stability at pH 8, demonstrated by the least amount of aggregation present in the pH 8 sample. The addition of salt to the gold conjugate was an effective test method for product A-D. Product A-C showed a difference in stability from pH 7-8, whereas product D was the only product that was stable at pH 7, pH 7.5 and pH 8.

UV-VIS results for product A showed that this gold colloid conjugate is the most stable in pH 8, demonstrated by the lowest absorbance at longer wavelengths, and the narrowest curve in the pH 8 spectrum. The UV-VIS results in **Figure 3** correlated with the salt testing results in **Figure 2**. Product D was the most stable at all three pH points and showed only a slight increase in stability at pH 8.

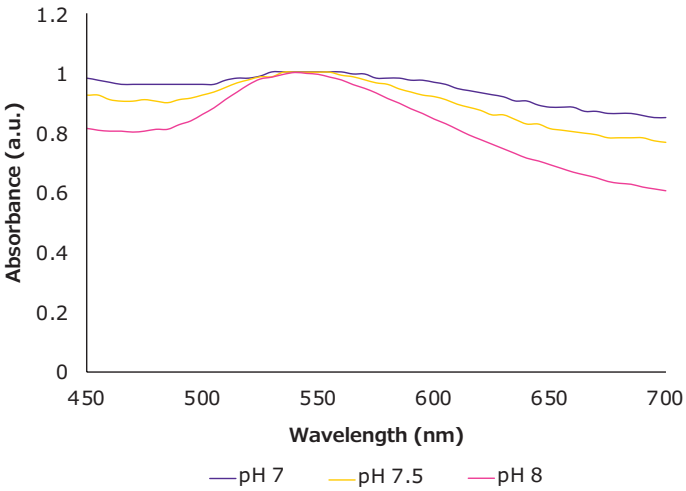
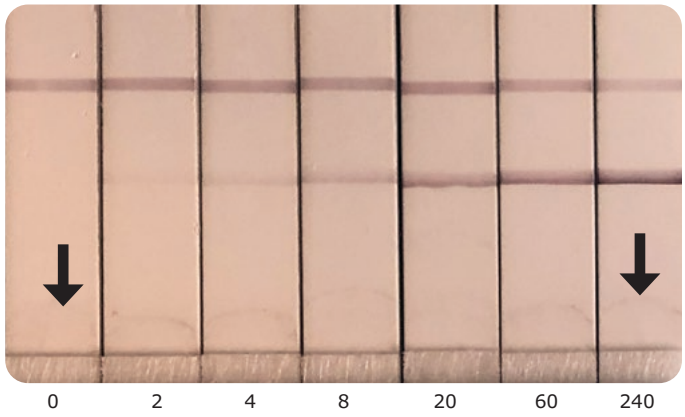
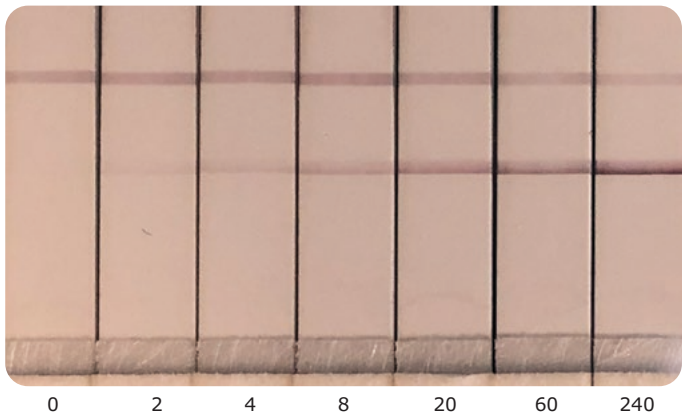


Figure 3 – UV-VIS spectra for product A after conjugation. a.u. represents arbitrary units. All spectra were normalised.

pH7



pH7.5



pH8

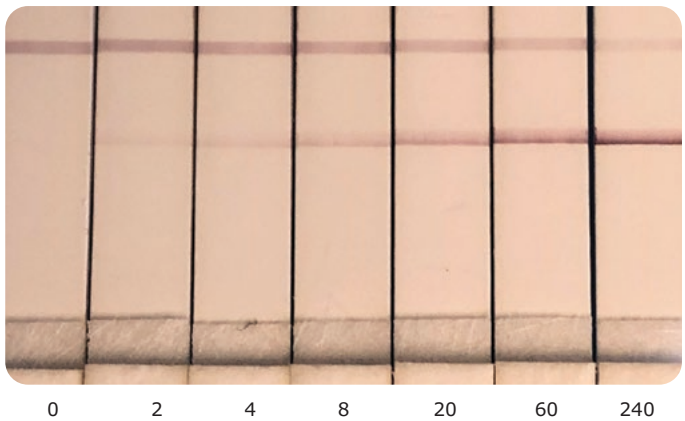


Figure 4 – Lateral flow strips for product A from pH 7-8. Arrows indicate the presence of aggregation on the strips. 0-240 on the bottom of the image represents the amount of HBsAg analyte added in nanogram/millilitre (ng/mL).

The lateral flow strips showed aggregation on the strips at pH 7 for product A. Product A-C all had aggregation present on the strips at pH 7.

Step 2 – Detector Antibody Concentration Testing

Following optimization of the pH for gold conjugates, the ideal pH was selected, and the concentration of detector antibody was varied to find the ideal antibody concentration. The concentrations below indicate approximately 10 - 120 microgram per millilitre (µg/mL) of detector antibody used per 1 mL of

product A gold colloid (equivalent concentrations per amount of gold colloid were used for the other gold colloid products).

The detector antibody concentration 10 µg/mL was too low for product A as seen by the presence of aggregates in the solution. Stability was seen from at least 40ug/ml as a consistent colour was observed from this minimum concentration. Product D was the only product to show a further increase in stability at 120 µg/mL, whereas product A-C followed a similar trend as shown in **Figure 5**.

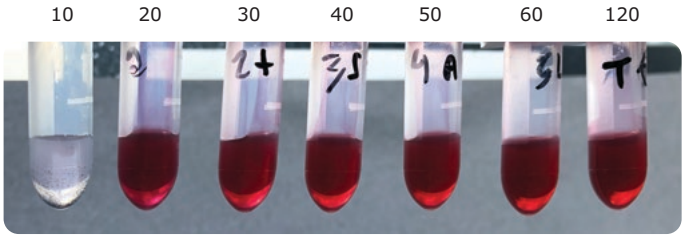


Figure 5 Images of product A at detector antibody concentrations 10 - 120 µg/mL after the addition of salt.

The UV-VIS result for product A show distinct differences in the stability of the conjugates from detector antibody concentration 10 - 120 µg/mL. Product D showed little difference in stability from 10 - 120 µg/mL with UV-VIS testing, however it should be noted that the supplier recommended the use of the salt test method. As such, product D did show stability differences with the addition of salt. This highlights the importance in following the recommended protocol from the supplier to find the technique most suited to the gold colloid product.

The lateral flow strip images show the sensitivity and specificity achieved for product A. There were no false positive results observed. All gold conjugates fully released from the conjugate pad at the higher antibody concentrations of detector antibody. However, there was aggregation present as indicated by the arrows. The arrows indicate aggregation present on the strips at the detector antibody concentration of 10 µg/mL. This gold colloid conjugate aggregation at low concentrations was seen for product A-C.

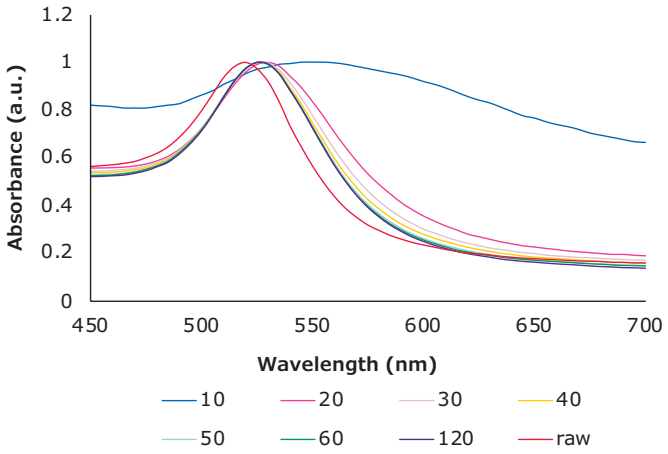


Figure 6 UV-VIS results for product A for detector antibody concentration 10 - 120 µg/mL. The "raw" sample represents unconjugated gold colloid. a.u. represents arbitrary units. All spectra were normalised.

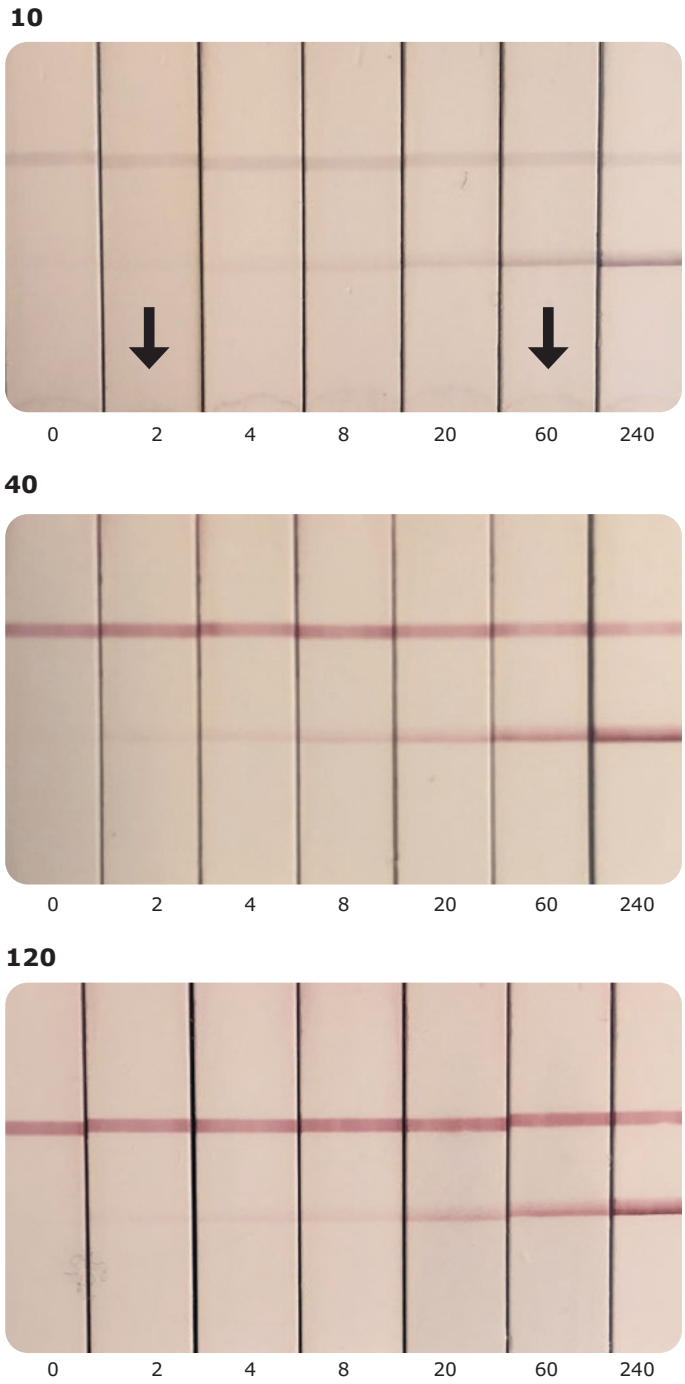


Figure 7 – Lateral flow strip images for product A at detector antibody concentrations 10, 40 and 120 µg/mL. 0 - 240 on the bottom of the image represents the amount of HBsAg analyte added in nanogram/millilitre (ng/mL).

Figure 8 shows the detector antibody concentration strips displayed beside each other when run with 4 ng/mL of analyte. This analyte concentration of 4 ng/mL was shown in isolation to demonstrate the decrease in sensitivity with an increase in detector antibody concentration for product A. This is clear when comparing the detector antibody concentration 20 µg/mL versus 120 µg/mL in **Figure 8**. Detector antibody at 120 µg/mL results in a less sensitive test line achieved in the lateral flow assay (LFA) than the detector antibody concentration of 20 µg/mL. This is an important consideration when choosing the optimal detector antibody concentration to proceed with in the assay. Product A-C followed this trend, whereas product D did not.

4 ng/mL HBsAg

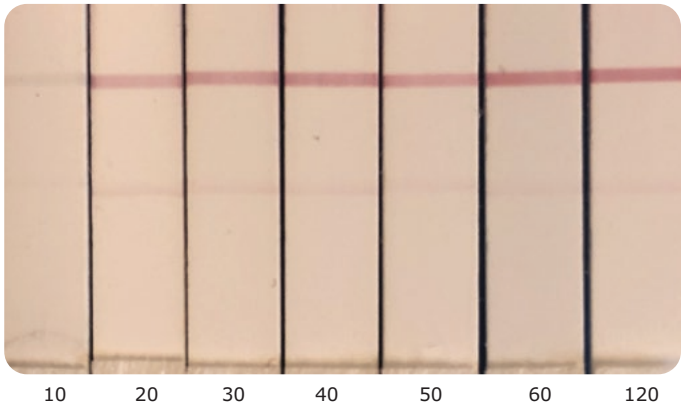


Figure 8 Lateral flow strip images for product A at the analyte concentration of 4 ng/mL. The numbers 10 - 120 represent the strips with the conjugate at detector concentration in µg/mL.

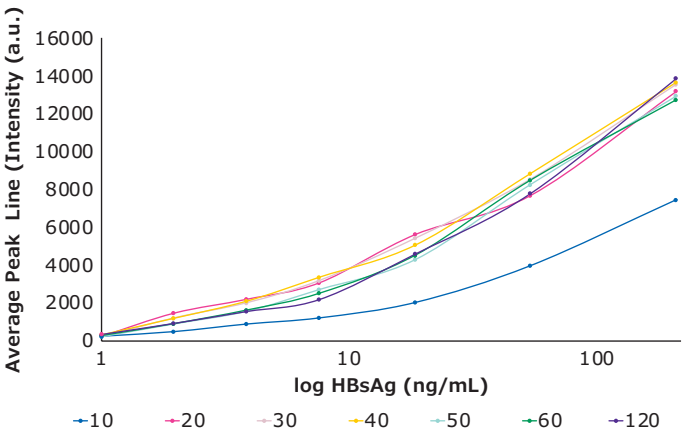


Figure 9 - Axxin AX-2X reader quantitative results from the strips from product A with detector antibody concentrations 10 - 120 µg/mL. The x-axis is in logarithmic scale (log) with the value 1 representing 0, as 0 cannot be plotted on a log scale.

Summary

Overall, all four gold colloids tested were of suitable quality to be used for passive conjugations for a lateral flow assay. Some conjugations were easier to optimise than others, as some suppliers had more detailed protocols, and gave additional information to assist with the experimental work. The above optimization steps demonstrated the unique properties of the gold colloids. Product D conjugate was relatively insensitive to changes in pH whereas product A-C conjugates were more stable at certain pH points. Furthermore, in the detector antibody concentration testing, product A-C conjugates showed a trend of having a decrease in sensitivity with an increase in detector antibody concentration. Product D conjugate displayed an increase in stability at the detector antibody concentration of 120 µg/mL with salt testing, whereas product A-C conjugates were stable at 40 µg/mL. Finally, product A-D conjugates produced a slightly different shades of red on the test and control lines. Product A conjugate displayed a bright ruby red colour, whereas product C's conjugate test and control lines were more purple in colour. This could be important for the end user, as an off-red colour may lead to confusion in interpretating the result of the LFA strip that indicates the presence of a red test and control line.

The unique properties of these gold colloids and how they perform under the optimization conditions are important considerations when choosing a gold colloid product for a passive conjugation. Some gold colloid products are more sensitive to pH changes than others, and some gold colloids require less detector antibody. Furthermore, each gold colloid required some level of optimization with the protocol given by the supplier. At Merck we offer assay development services, including conjugation protocol optimization, that can leverage our expertise to move a LF assay through to commercialization more efficiently.

Please visit our website for more information:
Lateral Flow Assay Development Services
sigmaaldrich.com/assaydev

Materials/Methods

All golds were tested, where possible, using the protocols specified by the supplier. This allowed the assessment of the protocols themselves, while also ensuring that the conjugate was made as per supplier instructions. Modifications to the protocol included changes in the gold colloid volume used at the beginning, buffer changes (all conjugates were made in phosphate buffers), and the inclusion of salt, UV VIS or lateral flow testing where only one technique was specified or where the technique specified could not be done in our laboratory.

The assay used here was a Hepatitis B Surface Antigen assay. The detector antibody concentration was varied while the capture antibody concentration was fixed. The strips were run using diluted human serum.

Materials	
Product Information	**Cat. No.**
Surewick C083 Cellulose Fibre Sample Pad	**CFSP223000**
Surewick GFDX Glass Fiber Conjugate Pad	**GFDX203000**
Hi-FlowTM Plus 135 Membrane, 2-mil backing	**SHF1350225**
Surewick C083 Cellulose Fiber Absorbent Pad	**CFSP223000**
Adhesive Backing Card	**HF000MC100**

References

1. Hermanson, Greg T. 2013. Bioconjugate Techniques, **Academic Press, London.**
2. Momeni, Arefe 2022. Gold-based nanoplatform for a rapid flow immunochromatographic test assay for gluten detection, **BMC Biomedical Engineering.**
3. Shengqiang Hu, 2020. Dissecting the Effect of Salt for More Sensitive Label-Free Colorimetric Detection of DNA Using Gold Nanoparticles. **Analytical Chemistry.**
4. Haiss, Wolfgang, 2007. Determination of Size and Concentration of Gold Nanoparticles from UV-VIS Spectra. **Analytical Chemistry.**

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