WHITEPAPER

BBI Solutions

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Effect of gold-colloid nanoparticle size

on sensitivity of lateral flow assays

A direct capture lateral flow assay (LFI) system was established using biotinylated rabbit IgG capture and Mab anti-biotin conjugates. Conjugates were manufactured using 20, 40, 60 and 80 nm gold nanoparticles. This system was used to investigate the relationship between conjugated gold nanoparticle size and achievable assay sensitivity.

Results indicate that, when test line intensities are read in the CAMAG reader system, sensitivity is enhanced as particle size increases from 20 through to 60 nm under equivalent conditions of capture concentration and conjugate OD. Peak sensitivity was achieved using 60 nm conjugates. 80 nm conjugates were the next most sensitive, ahead of 40 nm and 20 nm systems.

The data from this study suggest that the sensitivity gain due to using a larger conjugate size is marginal in visually interpreted assays. However, access to larger particle sizes adds value, even where no assay performance gain is achieved as the amount of conjugated antibody decreases relative to particle size, and hence the cost of materials.

INTRODUCTION

The purpose of this study was to compare the hierarchical range of sizes 20nm, 40nm, 60nm and 80nm of the colloids post conjugation looking for signal strength at known concentration of antibody. The rationale for this is that the gold colloids of particle sizes 20nm-40nm-60nm-80nm are all manufactured under the same methodology, using the same materials and conjugated with the same antibody (under optimal pH value and optimal concentrations of antibody) therefore any differences seen in signal strength should be attributable to particle size.

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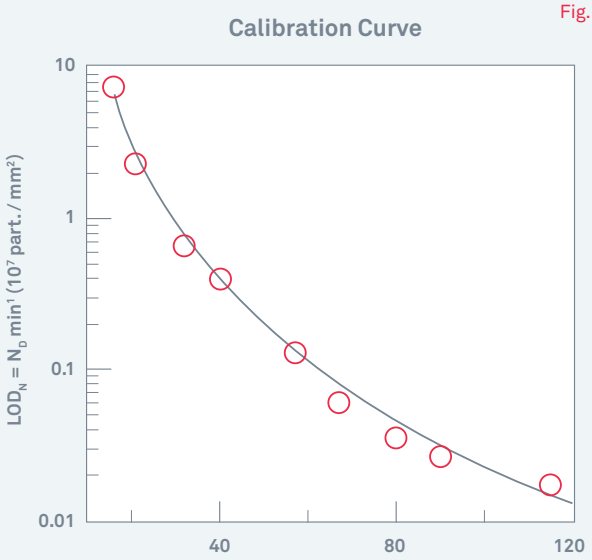
BACKGROUND

Published research (Khlebtsov et al., 2019; Serebrennikova et al., 2017) indicates that there is increased signal strength at low analyte conditions as particle size increases in a gold-nanoparticle-based LFI.

Fig. 1



Calibration curve for hierarchical nanoparticle diameter for the Limit of detection LOD (Khlebtsov et al., 2019)

INVESTIGATION

Methods

The following conjugates were manufactured. Conditions of pH and antibody loading were optimised for each particle size:

- **20nm Mab anti-biotin gold Conjugate**
- **40nm Mab anti-biotin gold Conjugate**
- **60nm Mab anti-biotin gold Conjugate**
- **80nm Mab anti-biotin gold Conjugate**

Nitrocellulose HF135 was striped on the Isoflow using biotinylated rabbit IgG at multiple concentrations of 0.500, 0.250, 0.125, 0.063, 0.032 and 0.016 ug/mL. A zero concentration buffer line was also striped. Striped nitrocellulose was laminated with an A222 upper wick and cut into 4 mm width dipsticks.

Conjugates were initially diluted to OD 2, 1 and 0.5 using PBS pH 7.2, 1% BSA, 1% Tween 20. A further concentration of OD 4 was subsequently prepared and tested for the 60 and 80 nm conjugates only.

Half dipstick testing was performed by pipetting 35 uL diluted conjugate into the wells of a microtitre plate. Half dipsticks (n=7 per condition) were placed in the conjugate and half dipsticks run until the wells are dry. Dipsticks were then transferred to wells containing 20 uL PBS 1% T20 washing buffer until the wells are dry.

> Photographs were taken of the test strips and test line intensities read using the CAMAG reader.

Results

The relationships between capture and conjugate concentration and sensitivity, and nanoparticle size and sensitivity are shown below. Test line intensity data was collected from the CAMAG reader, and photographs of the test strips were taken to record the corresponding visual appearance of the tests. Note that as absorbance spectra differ between particle sizes, the CAMAG was set to use the wavelengths below for each particle size.

- 80nm 555nm
- 60nm 520nm
- 40nm 520nm
- 20nm 520nm

This was for equivalence with the methods used for liquid conjugate OD measurement.

It should be noted that fixed conjugate ODs were used when comparing the different particle sizes. This is a relevant measure as it is the unit used to represent the concentration of stock BBI conjugates. It does not represent equivalent particle number, or binding capacity between conditions, however. The value of establishing standard performance using these measures is discussed in the conclusions.

OD 2 is the highest conjugate concentration tested for all particle sizes. Comparison of each system at OD 2 illustrates relative achievable sensitivity when particle size is the only variable and test strips are read in the CAMAG.

Comparison of CAMAG data with the visual appearance of the test strips suggests that there is greater potential to achieve improved sensitivity by increasing particle size in a reader-based assay than in a visually interpreted test. The visual appearance of the 40 and 60 nm conjugates is much more similar than is suggested by the CAMAG data. 80 nm conjugates appear equivalent to or slightly less intense than the corresponding 60 nm conjugate data. A subtle hue-shift is also associated with increasing particle size. This may suggest that particle-size specific optimisation of the reader optics is an important consideration for assay development where results are to be read from a reader.

Fig. 2

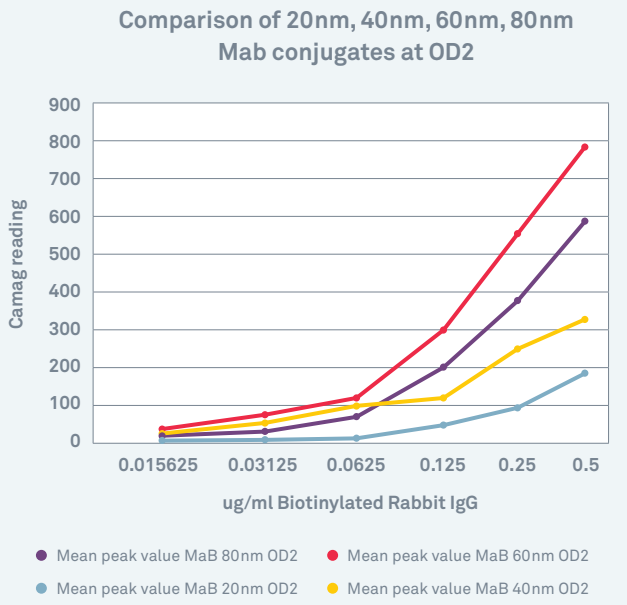
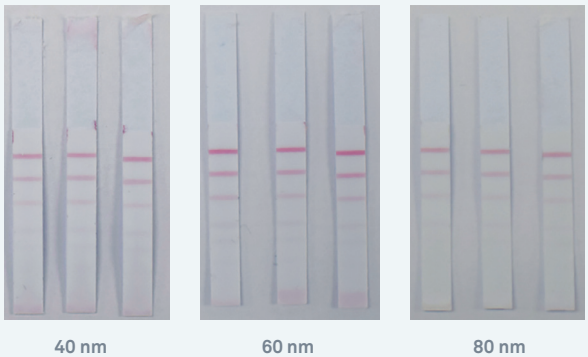


Fig. 3



OD 2 is the highest conjugate concentration tested for all particle sizes

CONCLUSION

The data from this study indicates that increased particle size of a conjugate does improve signal strength from 20nm to 40nm to 60nm while we saw a drop of at 80nm relative to the 60nm but still an improved signal strength over 40nm, but this may be because of the nature of the peak shape from 60nm upwards and the limitations of reading compared to the human eye.

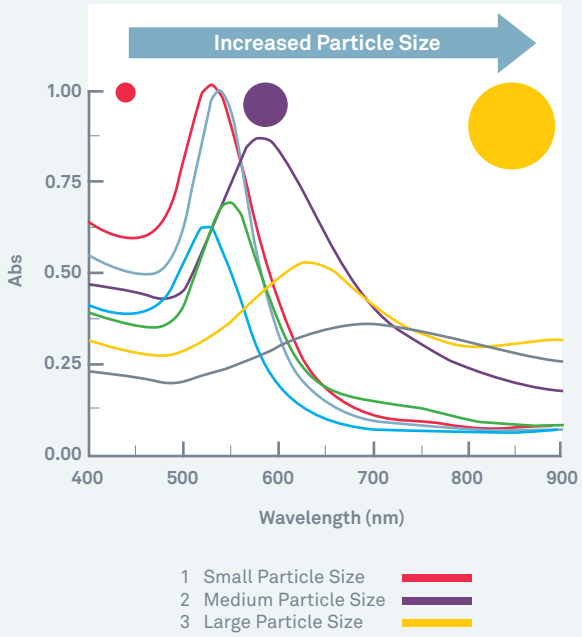
Figure 4 shows that hierarchical large gold nanoparticles display contrasting colour, which improves interpretation (visually and quantitatively). From the wavelength profiles in Figure 4 the peaks can be seen to become increasingly spread out and flatter as the particle size increases.

This can make OD definition more problematic as the reader system used for this study only reads at a specific wavelength and as such relatively more of the signal will not be read as the peaks widen and flatten with increased particle size. Therefore, the reader may return lower values than the actual visual return seen by the human eye. to be read from a reader.

This study has shown the benefits in signal strength enhancement utilising larger sized conjugate gold nanoparticles, therefore when developing a lateral flow assay, it is important to assess a range of gold nanoparticle sizes as part of assay feasibility and optimisation stages to ensure assay performance is optimised to deliver the best performance .

Fig. 4

Particles size and peak profile



BBI Solutions gold nanoparticle range for rapid diagnostic assays

	20nm Gold Nanoparticles	40nm Gold Nanoparticles	60nm Gold Nanoparticles	80nm Gold Nanoparticles
Average Diameter	19.0 – 21.0nm	37.0 – 43.0nm	57.0 – 63.0nm	77.0 – 85.0nm
Maximum acceptable %CV	8%			
Number of odd shapes per 100 particles	≤5			≤10
Optical density @520nm (using a 1cm pathlength)	• Bulk available at OD 1 • Up to 50mL available at OD 5/10	• Bulk available at OD 1 • Bulk available at OD 4.5-5.0 • Up to 50mL available at OD 10	• Bulk Available at OD1	• Bulk available at OD 1.0 at 555nm
Batch scale	Up to 100L at OD 1	Up to 340L at OD 1	Up to 64L at OD 1	Up to 64L at OD 1
Capping agent	Citrate			
Presentation matrix	Suspended in H_2O, no preservative			
Shelf life	15 months from date of manufacture	12 months from date of manufacture		
Storage	2 – 8°C – do not freeze			