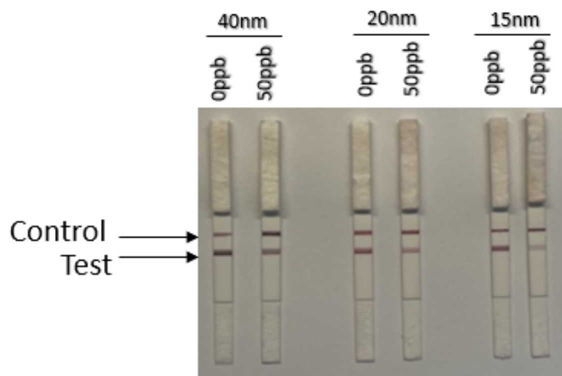
The Impact of Colloidal Gold on Lateral Flow Optimization

Introduction:

Lateral flow tests have many components that influence their behavior (i.e., run time, detection limits, reliability, robustness, stability, ease of use). These include, but are not limited to, the colloidal gold, sample pad, conjugate pad, nitrocellulose, wick, running buffer, backing and antibody. When developing a lateral flow assay it is important to lock down the optimal reagents for a particular need. One of the core reagents manufactured by Attogene is the colloidal gold spheres applied in the construction of lateral flow assays. Here we offer some insights into colloidal gold optimization.

Results and Conclusion:

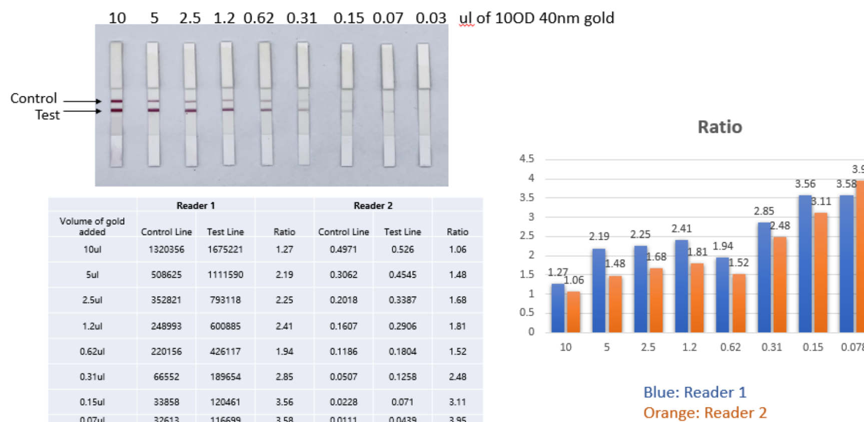
One important component that influence lateral flow assay behavior is the size of the colloidal gold particles. Here, we tested three different antibody conjugated gold particles with sizes of 15nm, 20nm, and 40nm on a competitive lateral flow assay. Colloidal gold of the indicated sizes were conjugated with an anti-drug mouse monoclonal antibody using previously optimized conditions. Briefly, conjugation was performed by mixing the gold with the anti-drug antibody in optimal buffering conditions, blocked and concentrated to 10 OD by centrifugation. Next, nitrocellulose backed cards were sprayed with goat anti-mouse (control line) and the Drug-BSA (Test line) using the BioDot XYZ. Cards were laminated with a wick and conjugate pad both having 4mm overlap with the nitrocellulose. We next cut the cards into 4.5mm strips and performed a test where running buffer was mixed with 10µl of 10OD anti-drug mouse monoclonal antibody conjugated gold of the indicated size in wells of a 96 well plate either lacking or containing 50ppb of the free drug. As seen in Figure 1, while we found that while 40nm gold gave a brighter signal intensity as



Concentration of Competitor Drug	Gold Used	Control Line	Test Line	Ratio
0ppb	40nm	887462	1603222	1.81
50ppb	40nm	1554254	929304	0.6
0ppb	20nm	1132052	1137574	1
50ppb	20nm	1172347	491208	0.42
0ppb	15nm	994258	1161122	1.17
50ppb	15nm	1132219	217390	0.19

Figure 1. Gold conjugates of 15nm, 20 and 40nm containing anti-drug mouse monoclonal antibody were mixed with running buffer lacking or containing 50ppb of free drug. Strips were run for 15 minutes after which they were analyzed using a lateral flow reader. Results for the test, control and ratio are shown in the data below the picture.

indicated by eye and from the lateral flow reader results, the 40nm particles were less clear when you evaluate the impact of free drug competition on the assay. As seen in Fig. 1, the 20 and 15nm size gold particle conjugates were far superior when used to evaluate the impact of free drug competition by eye. These data indicate that gold particle size can influence the visual performance of a lateral flow assay and thus is an important variable to consider when developing your assay.



	Reader 1			Reader 2		
Volume of gold added	Control Line	Test Line	Ratio	Control Line	Test Line	Ratio
10ul	1320356	1675221	1.27	0.4971	0.526	1.06
5ul	508625	1111590	2.19	0.3062	0.4545	1.48
2.5ul	352821	793118	2.25	0.2018	0.3387	1.68
1.2ul	248993	600885	2.41	0.1607	0.2906	1.81
0.62ul	220156	426117	1.94	0.1186	0.1804	1.52
0.31ul	66552	189654	2.85	0.0507	0.1258	2.48
0.15ul	33858	120461	3.56	0.0228	0.071	3.11
0.07ul	32613	116699	3.58	0.0111	0.0439	3.95

Figure 2. 40nm gold conjugate was mixed with running buffer and serially diluted to determine the concentration of gold which would generate a robust signal while also dialing back the visual intensity of the test and control lines. The strips were read in two lateral flow readers and the ratio of the tests were compared in a graph located to the right.

To test this hypothesis, we next performed a dilution series of the 40nm colloidal gold conjugate starting with 10µl of 10OD gold down to as little as 0.03µl of 10OD gold particles (Figure 2). We also compared the readings obtained from this dilution series using two different lateral flow readers. From these studies, we found that decreasing the concentration of gold used in the test enhances the differences between the test and the control lines. For example, the control line in this test is less intense than the test line because the test line and control lines both compete for the anti-drug mouse monoclonal antibody attached to the gold. So as the conjugate runs over the nitrocellulose, it binds the test line first then any leftover gold which has not associated with the drug conjugate test line will be captured by the control line (which contains goat anti mouse IgG). It is interesting to note that at lower gold concentrations, ratio values from the readers begin to expand up to nearly 4-fold difference while at high gold concentrations the ratio can be as low as 1 (i.e. equal test and control line) intensities. While the two readers provide very different readouts for the line values, it is also important to note that they both provide generally similar ratio values. Finally, decreasing the gold in half from the starting concentration does not lead to a decrease in values in the control and test line values – indicating that the assay is saturated at these levels.

Based on the information from Figure 2, we chose two concentrations of gold to repeat the drug titration to determine if we can enhance the visual impact of the test line in the 40nm gold particle assay. As seen in Figure 3, we added either 5µl or 2.5µl of 10OD colloidal gold particles of the indicated size into wells either lacking or containing 50ppb of free drug. The results in Figure 3 demonstrate that dialing back the

amount of 40nm gold had minimal impact on the visual reading but was still less pleasing to the eye as the results that were obtained with the smaller gold particles.

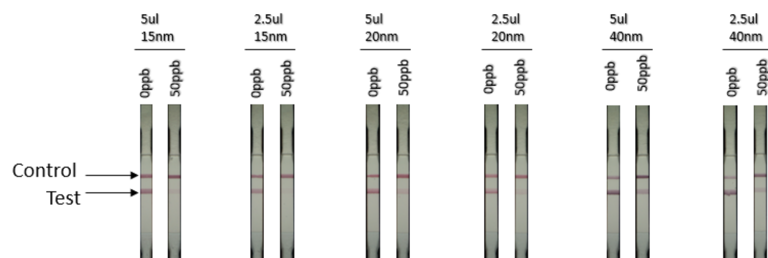


Figure 3. Either 5µl or 2.5µl of gold particles of the indicated size were incubated with lateral flow running buffer either lacking or containing 50ppb of free drug and assays were run as described above.

Conclusions:

Publications demonstrating new lateral flow techniques most frequently use multiple different assay components. This really makes it difficult to diligently compare one technique relative to another. In our experience, it is important to note several key factors in developing highly effective lateral flow assays. First, the design should always be locked down to provide the consistency desired in a robust test. It is important, for example, to use colloidal gold of high consistency and purity with conjugates made using consistent techniques. Second, the choice of a reader can influence readings and interpretation of a test. Therefore, tests should be built and validated with either a visual interpretation in mind or using one specific instrument. For example, when the amount of gold is reduced in each well by 50%, the relative line intensity does not necessarily drop exactly in accordance with the decrease in gold particles. The ratio of the two lines, however, would be expected to stay somewhat similar in the linear range of the detectability of the test. We saw in Figure 2 adrift in ratio of the two lines with both readers in the lower range of gold particle concentrations as the test line becomes may be considered a shadow line. It is hard to make the test line disappear in an assay even at very low concentrations of gold. In conclusion, the concentration of gold, size of the gold particle and the specific reader that is used can all impact the readability of your lateral flow assay and should be well optimized to provide and ensure consistent and robust results.