Analysis of Stoichiometry of Reagents Using Attogene's Universal Lateral Flow Assay Kit

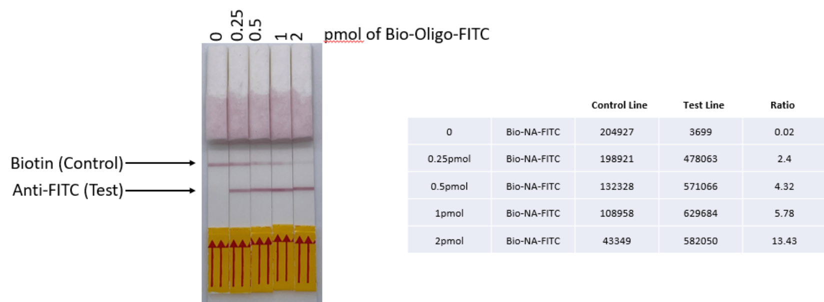
Introduction:

Using Attogene's Universal Lateral Flow Kits for the development of assays has become an important tool for many researchers – in particular for those lacking the expensive equipment and/or expertise it takes to effectively generate conjugates and sprayed lateral flow strips. Attogene has developed key materials to support this growing field using our internal lateral flow manufacturing equipment and ingenuity. The key ingredients of our kit include specialized colloidal gold that is embedded into conjugate pads and applied to cards containing nitrocellulose adaptable test and control lines. We have used these key reagents to analyze the impact of stoichiometry of many tagged analytes on the test and control line intensities. Our results clearly document the importance of stoichiometry in lateral flow assay design and development.

Results and Conclusion:

Research efforts exploring lateral flow-based tests have been limited by the need for expensive instrumentation which is used for spraying nitrocellulose membranes with biological materials and evenly cutting cards into strips. To enhance the exploration of the field of lateral flow development, Attogene has released numerous standardized materials including colloidal gold, colloidal gold conjugates, cassettes, and critical lateral flow assay running buffers. In addition, we have recently launched the Universal Lateral Flow Assay Kit (https://www.attogene.com/shop/universal-lateral-flow-assay-kit/) that contains strips composed of streptavidin gold embedded into conjugate pads applied to cards containing a nitrocellulose membrane sprayed with biotin and anti-FITC capture lines. In order to demonstrate the accuracy and utility of our kit, the dipsticks contained in the Universal Lateral Flow Assay Kit were used in a stochiometric study with a synthetic nucleic acid containing a 5' biotin and 3' FITC made by Integrated DNA Technologies. To analyze stochiometric effects, the biotin and FITC labeled nucleic acid was mixed at increasing concentrations with 150µl of a proprietary specially designed lateral flow running buffer in a well of a 96 well plate. The dipstick from the Universal Lateral Flow Assay Kit was added into the well and run for 10 minutes (Figure 1).

The data shown in Figure 1 demonstrate the importance of the relative amounts of molecules of a synthetic dual labeled nucleic acid with the amount of gold particles to uncover optimal line intensities. The biotin sprayed line captures streptavidin gold when streptavidin in not occupied with the dual labeled oligonucleotide. However, as more dual labeled nucleic acid is added, the binding to the anti-FITC reaches maximal intensity (1pmol) and then diminishes due to over saturation with the dual labeled nucleic acid. At 4pmoles (data not shown), free biotinylated nucleic acid overtakes the system to bind both the



		Control Line	Test Line	Ratio
0	Bio-NA-FITC	204927	3699	0.02
0.25pmol	Bio-NA-FITC	198921	478063	2.4
0.5pmol	Bio-NA-FITC	132328	571066	4.32
1pmol	Bio-NA-FITC	108958	629684	5.78
2pmol	Bio-NA-FITC	43349	582050	13.43

Figure 1. Lateral flow dipsticks containing streptavidin gold conjugate applied to strips containing anti-FITC and Biotin lines. The indicated amounts of a dual-labeled oligonucleotide were added, and strips were analyzed using a lateral flow reader.

streptavidin gold and anti-FITC while also occupying every gold particle in the system leading to a loss in intensity of both the biotin line and the anti-FITC line.

Next, we evaluated Attogene' s streptavidin colloidal gold with cards containing anti-rabbit IgG and biotin along with a Control Line (biotin) and a Test Line (Goat Anti Rabbit -GAR). Then 10µl of 10OD Attogene's streptavidin gold was mixed with varying concentrations of biotinylated rabbit IgG in 150µl of our specialized lateral flow running buffer and applied to the sample port of cassettes.

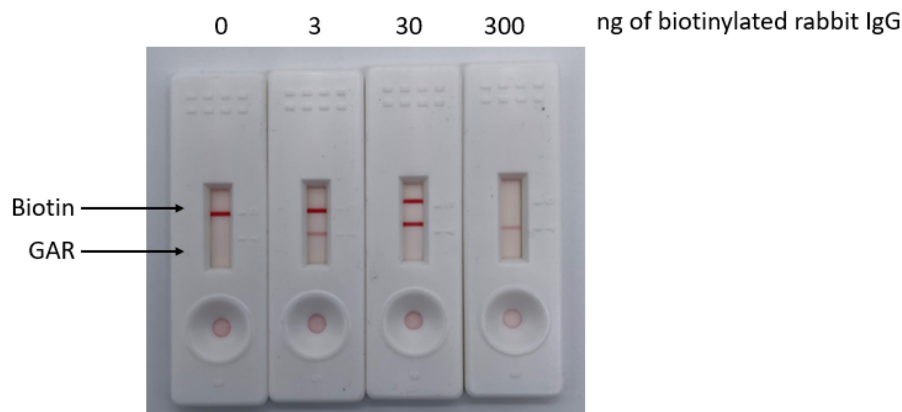


Figure 2. Streptavidin colloidal gold conjugated was applied to cards containing Goat anti rabbit (GAR – Test Line) and biotin (control line). 10μl of Attogene's 10OD streptavidin gold was mixed with the indicated varying concentrations of biotinylated rabbit IgG.

The data in Figure 2 demonstrate the importance of the relative amounts of molecules of biotinylated rabbit IgG to the amount of gold particles in order to find optimal line intensities. The Biotin line captures streptavidin gold when streptavidin is not fully occupied with biotinylated rabbit IgG. When more biotinylated rabbit IgG added, the binding to the goat anti rabbit line (GAR) reaches maximal intensity (30ng) and then diminishes due to over saturation with biotinylated rabbit IgG. At 300ng, free biotinylated rabbit IgG overtakes the system to bind both the streptavidin gold and GAR while also occupying every gold particle in the system leading to a loss of both the biotin line and the GAR line.

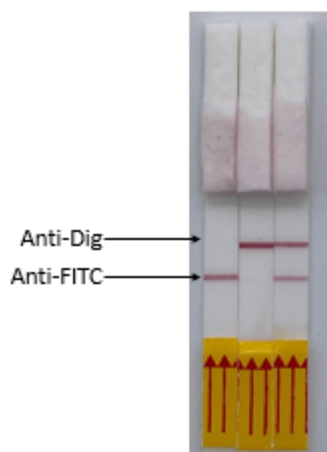


Figure 3. Cards sprayed with anti - FITC and anti-DIG were assembled with a conjugate pad containing Attogene's streptavidin gold. Oligonucleotides containing just biotin and FITC (left), Biotin and Dig (middle) or both oligos together (right lane) were applied to the running buffer and run on the dipsticks.

Finally, we next evaluated the impact on the assay of mixing two biotin labeled oligonucleotides at specific stoichiometric concentrations on lateral flow strips. One nucleic acid was labeled with biotin and FITC and the second was labeled with biotin and DIG. Strips contained both anti-FITC and anti-Dig antibody. Because both nucleic acids are both competing for the streptavidin binding sites on the colloidal gold, the ratio of the two is important to allow effective intensities to be visualized on both the anti-FITC and anti-Dig lines. As seen in **Figure 3**, the oligonucleotides were applied at 1pmol of each individually or 1pmol of each together. Importantly, both oligos could be effectively detected at this concentration (right lane).

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Conclusions: Stoichiometry of reagent binding sites in the lateral flow assay are important to balance for optimal performance. Ratio adjustments may be needed for careful adjustments of intensity of the lines. These can be influenced by running buffer, blocking, concentration, stability, and affinity of each analyte sprayed onto nitrocellulose. Attogene is always here to help you with your assay optimization needs.

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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