Lateral Flow: Making Magnetic Particles A Viable And Easier To Use Replacement To Gold Nanoparticles.

Introduction

While lateral flow immunoassays represent a quick, low cost, easy to use alternative to conventional clinical testing, a do-it-yourself approach to assay development in the lateral flow format is generally hampered due to the costs of raw materials or uncertainties in the handling of lesser documented materials[1]. The majority of current lateral flow tests utilise gold nanoparticles as the primary detection label. Advances have been made in the use of alternative detection strategies, such as magnetic particles, but there has been limited uptake for example, by IVD manufacturers[2].

Anteo's coupling kit for lateral flow looks to simplify this process of screening particle compatibility by using polymeric metal complexes to anchor the proteins of interest to the particle. The process is much simpler and less time consuming than the current conventional chemistries and requires less antibody to achieve relative performance in passive couplings due to improvements in both antibody orientation and functionality. This in turn leads to the generation of monodispersed, highly stable and functional magnetic particle conjugates for lateral flow applications.

This application note outlines the preparation of 200nm magnetic particles conjugates for use in an anti-hepatitis B surface antigen based lateral flow assay.

Summary

The utility and flexibility of Anteo's surface chemistry has given rise to a number of different kits focused in the areas of antibody coupling, magnetic bead separation and lateral flow. Using patented metal chelation chemistry to coat the particle surface, Anteo have created an easy-to-use product that eliminates the need for volatile chemical preparation while producing a functional particle surface, ready for conjugation.

The kit contains all the essential components to activate your chosen particle with Anteo's activation chemistry and the complementary buffers to ensure functional binding of your protein of interest. The flexibility of being able to use particles of various compositions and different surface groups coupled with relative ease of use allows for the rapid screening of compatible surfaces with high reproducibility and at low costs. The kit also provides an easily accessible entry point for next generation development of novel conjugates for lateral flow.

Materials and Methods

Lateral Flow Coupling Kit (Cat# A-VMPLFMP-10)
- Particle Pretreatment Solution
- Particle Activation Solution
- Particle Intermediate Solution
- Coupling Buffer
- Blocking Buffer
- Storage Buffer

Antibody Coupling
- AllMag SuperParamagnetic Nanoparticles, 200nm (Allrun, Cat # PM3-020)
- Monoclonal mouse anti-hepatitis B virus surface antigen: Hs41 (HyTest, Cat # 3HB12)

Lateral Flow Assay
- Half dipstick (assembled in-house), antibody lines at 1mg/mL:
- Test line: Monoclonal mouse anti-hepatitis B virus surface antigen: Hs33 (HyTest, Cat # 3HB12)
- Control line: Goat anti-mouse IgG (H+L) (Jackson, Cat # 115-005-003)
- Running buffer: 50mM tris buffered saline + 1% Tween 20 pH 8.0
- Sera human (Sigma, Cat # S7023)

Magnetic Particle Activation
A sample of stock particles (particles @ 10mg/mL), was placed on a magnet until the particles separated from the solution. Supernatant was removed and replaced with pretreatment solution to form a 10x concentrate solution (particles at 100mg/mL). Particles were then added to the activation solution (particles at 10mg/mL), resuspended using vortexing and sonication and incubated at room temperature for one hour under gentle end over end rotation mixing. Activation solution was aspirated and replaced with the intermediate solution.

Antibody Coupling and Blocking
Activated particles were removed from the imtermediate solution and resuspended in coupling buffer. This wash step was repeated twice more (total of three washes).

In a new tube, the antibody solution was prepared at double the required concentrations (e.g. for a 200nm particle, the antibody concentration was titrated around the 100ug / mg of beads - 120ug/mg beads). Washed particles were then added to the antibody solution (particles @ 5mg/mL) followed by vortex and sonication before leaving the tube to incubate at room temperature for one hour under gentle end over end rotation mixing.

Following the incubation period blocking buffer was added to the particle solution, followed by vortex and incubation with end over end rotation for a further hour. Particles were then separated from the solution, by supernatant aspiration and then resuspension with storage buffer. Particles were washed again before resuspending at the starting particle concentration (particles at 10mg/mL). Vortex and sonicate the particles prior to further use.

Lateral Flow Assay
Using anti-hepatitis B surface antigen as an example the following steps outline the lateral flow assay method for particles coupled using Anteo's coupling kit. The assay results generated in this example was through the use of 10% human serum.

Hepatitis B antigen (HBsAg) was diluted to the required concentrations in 20% human serum (final concentration 10%) diluted with 50mM tris buffered saline + 0.5% Tween20 (TBST). The anti-HBsAg coupled magnetic particles were vortex and sonicated prior to dilution to 0.2mg/mL in TBST. A 25µL sample of each concentration of HBsAg was placed into separate wells of a Low-Bind 96 well plate (in triplicate) followed by addition of a 25µL aliquot of conjugate particles to each well.

Antibody dispticks (Test Line: anti-HBsAg antibody, Control Line: Goat anti mouse IgG) were added to each well and run until completion (roughly 10 minutes). A separate wash step may be implemented (25µL TBST) if required to reduced background before reading. Dipsticks were visually inspected before placing into a reflective dipstick reader to generate a digital measurement (Qiagen - ESE Quant LR3 reader).

Results

The following results were taken using a Malvern Zetasizer Nano ZS to confirm monodispersity of coupled particles, while a Qiagen - ESE Quant LR3 reader was used to read the peak reflectance data of the test lines on the lateral flow strips.

Figure 1 outlines the average size, polydispersity index (indicator of monodispersity) and zeta potential (surface charge) of the 200nm particle at key points during the coupling process. As indicated by the results the average particle size increased as subsequent layers of metal polymer, antibody and blocker were coupled to the surface. Little change was noted in the polydispersity index (PdI) indicating that there was no overall increase in particle aggregation. The change observed in the zeta potential from negative to positive is a standard indicator of surface activation by the Anteo metal polymer. The change back to a negative charge confirmed the complete coverage of the surface by antibodies and blocker.

Figure 1: Zetasizer size and surface potential measurements of the 200nm particle at intermediate steps.

Stage	Size (Measurement)	PdI	Zeta Potential
Bare Particle	196.8 nm	0.150	-37.9 mV
Surface activated	224.4 nm	0.118	+43.5 mV
Antibody coupled and blocked	284.2 nm	0.128	-34.3 mV

Figures 2 and 3 summarise the test line intensity data obtained through the use of a reflectance based strip reader in 10% human serum. From the raw data HBsAg sensitivity was observed to be at 1 - 2ng/mL while a limit of detection (LoD) calculation based on the 0ng/mL value plus three times the standard deviation (0 + 3x SD) estimates the sensitivity to be at 0.2 - 0.5ng/mL. Figure 4 shows the dipsticks for each antigen concentration. By visual observation, 1ng/mL displays greater test line intensity than the 0.5ng/mL and 0ng/mL concentrations which displayed some degree of non-specific binding.

Figure 2: ESE reader values of half dipstick lateral flow tests run in triplicate.

HBsAG	AVERAGE	STDEV	%CV
20	317	9.99	3.15%
10	212	20.57	9.72%
5	142	8.38	5.92%
2	88	10.60	12.10%
1	65	14.45	22.12%
0.5	56	18.76	33.80%
0	42	4.88	11.52%

Figure 3: Plotted data of the HBsAg lateral flow assay

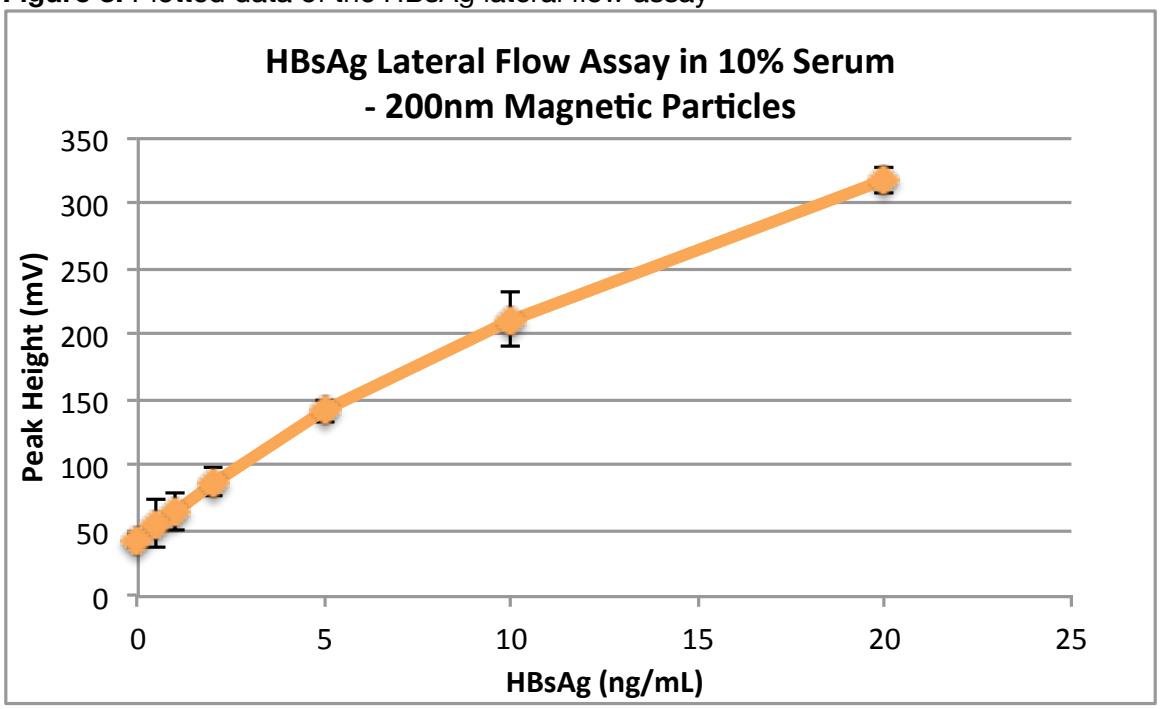
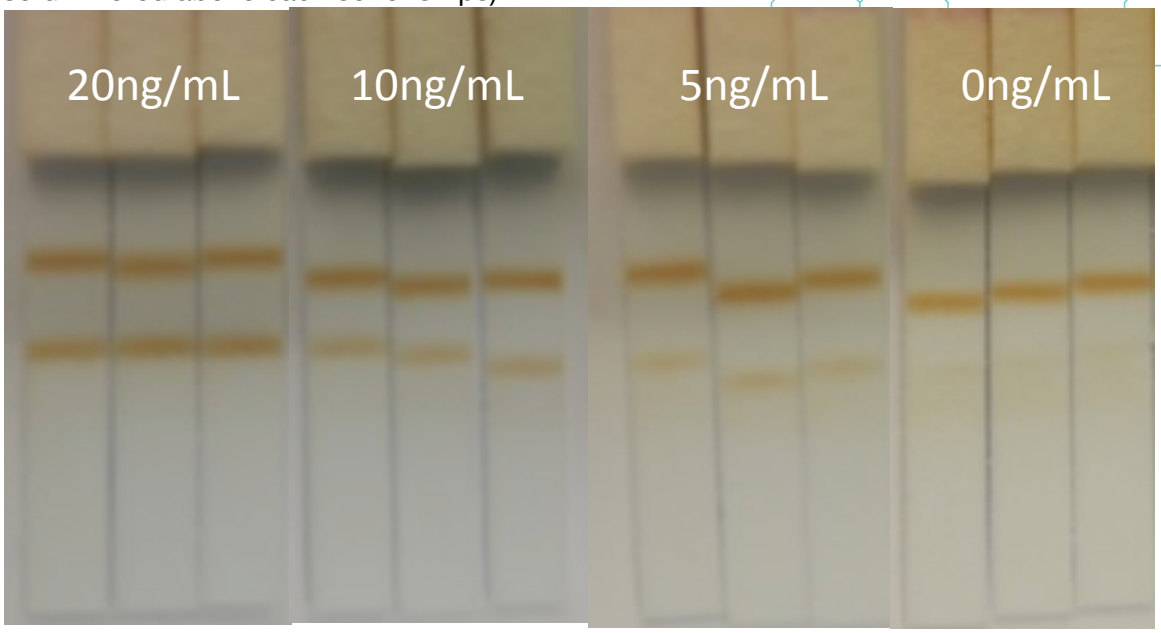


Figure 4: Selection of HBsAg lateral flow strips in triplicate (antigen concentrations in 10% human serum noted above each set of strips)



Discussion

Using the standard procedure supplied with Anteo's lateral flow coupling kit 200nm magnetic particles were coupled with anti-HBsAg monoclonal antibody. Zetasizer data confirmed monodisperse particles at the activation and coupling steps while changes in particle surface charge were characteristic of a complete coating of metal polymer and subsequent coverage with antibodies and blocker.

When assayed in a half dipstick lateral flow configuration the coupled particles detected antigen at the 1 - 2ng/mL concentration. Further optimisation of both the particle and/or the assay system would improve the overall assay sensitivity. It would also be possible to change from a reflectance or colorimetric-based detection method (in which only the surface particles are read) to that of a magnetic reader (where a change in applied voltage across all the captured particles may be measured)[3].

Commercial gold-based HBsAg lateral flow tests in full serum display detection limits of 2ng/mL[4], making the obtained results using Anteo's lateral flow coupling kit a viable starting point for further optimisation. Also by avoiding the recommended pH modifications according to the antibody pI commonly seen in gold-based couplings fine-tuning of the optimal antibody coupling conditions is greatly reduced in Anteo's procedure.

Conclusion

Using Anteo's coupling kit, magnetic particles of an appropriate size for lateral flow applications were successfully coupled with antibody and run in a standard lateral flow setup.
The ease of use of Anteo's kit accounts for the quick and effective production of particle samples while minimising hands on time and eliminating the use of volatile chemicals. This in turns allows the user to

rapidly screen potential candidates during the assay development cycle while providing a greater degree of flexibility and reproducibility not available to the current chemistries. While some of these improvements come down to a change from gold colloids to magnetic particles other problems may be introduced. Anteo's easy to use surface chemistry limits these inherent limitations in the current technologies and provides a platform for further development.

Contact Customer Support: If you have any questions about the Anteo Lateral Flow Coupling Kit, contact support@anteotech.com or call +61 (0) 7 3219 0085.

Anteo's products are for research use only.

References

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