



Lateral Flow

Achieving **5 x More Sensitivity**
and **Less Variation in Results** than
Covalent Chemistry with Magnetic Particles

by Anteo Technologies

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Summary

A new method of conjugating antibodies to particles was compared to covalent conjugation in a lateral flow assay for human Chorionic Gonadotropin (hCG). Anteo Technologies will be launching a new Lateral Flow Coupling Kit at AACC 2016 that contains Anteo's patented Activation Reagent with all necessary buffers for conjugating antibodies to a range of particles used for lateral flow applications. Here we demonstrate the performance advantages of using activated magnetic particles, only requiring the addition of antibody solution and blocking. Keeping all other variables constant, this kit was directly compared to covalently (EDAC chemistry) conjugated magnetic particles of the same type using visual detection as the readout. In this hCG study, this coupling kit achieves five times more sensitivity than the covalently conjugated magnetic particle based assay using half the amount of antibody.

Comparison of Anteo's Activated Magnetic Particles and Covalently Conjugated Magnetic Particles in a Lateral Flow Assay for Human Chorionic Gonadotropin (hCG)

Introduction

Lateral flow immunoassays (LFIA) or strip tests are conceptually simple immunoassays well suited to Point of Care (PoC) applications requiring little or no infrastructure support (Wong and Tse 2009, Sharma et al 2015).

In its original conception, the test is easy-to-use, relatively fast and low cost, giving good qualitative information where a simple yes/no answer is sufficient.

However, this same test system has been evolving to compete with more traditional laboratory based platforms. To achieve the requirements of greater assay accuracy and sensitivity without losing its basic advantages for PoC conditions, the actual strip test is now at the core of instruments such as hand held analysers comprising sample handling, reading and analysis modules. In brief, LFIA is no longer at the cheap and simple end of the spectrum in terms of diagnostic technologies (Sharma et al, 2015).

The above trends have resulted in continuous improvements over various materials and reagents used in LFIA of which Anteo Technologies' Lateral Flow Coupling Kit is one new development. Historically, gold colloids have dominated in LFIA with visualisation of conjugated gold colloids as they were captured on pre-defined lines on the test strip.

Passive conjugation of antibodies on gold colloids is conceptually simple but depending on the antibody, can lead to difficulties such as poor Ab functionality and loss of colloidal stability resulting in the formation of aggregates (Wong and Tse 2009). These difficulties change with scale, and achieving good uniformity during scale-up can often be very challenging.



Using 200 nm Magnetic Particles

The use of 200 nm magnetic nanoparticles activated with Anteo's reagent for lateral flow as an alternative to colloidal gold addresses a number of potential problematic situations encountered in the development of new lateral flow assays. For example, preparation of labelled conjugates is simplified using magnetic particles as they are easy to separate during conjugation, washing and blocking steps, with the additional benefit of inclusion of a magnetic label for performing magnetic immunoassays (MIA) (Shi et al 2015).

Alternatively, when used in conjunction with sufficiently dark magnetic particles visual detection alone can produce results rivalling those of other coloured particle types. The kit contains Anteo's Activation Reagent that can be used to activate particle sizes from 200 nm to 1 μ m that become immediately reactive to antibodies once Anteo's reagent is applied.

Conventionally, conjugation of antibodies to magnetic particles is commonly done by covalent chemistry using reagents such as 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (S-NHS). A reactive ester is formed on the magnetic particles, which reacts with amino groups on the protein.

Chemically, the reaction is simple but fraught with multiple variables to consider when trying to achieve optimum results. Conditions that increase stability of the ester during synthesis make it less reactive to amino groups on the protein. Increasing ester reactivity leads to side reactions. While different approaches can mitigate many such problems, these methods require pre-activation of particles which cannot be stored for any length of time.

The magnetic particles activated by Anteo's Activation Reagent are claimed to be stable in storage and highly reactive to proteins with other advantages such as ease of use, less antibodies used, faster conjugation and reduced aggregation amongst its benefits.

Using a hCG lateral flow assay previously developed using colloidal gold at Diagnostic Consulting Network (DCN), the Anteo coupling kits were compared to covalently conjugated magnetic particles using the same assay materials (membrane, pads, and antibodies) with the exception of the gold colloids.



Method

The hCG assay has previously been developed using colloidal gold at Diagnostic Consulting Network (DCN), Carlsbad, CA for internal use and demonstration purposes. The same assay materials (membrane, pads, and antibodies) were used with Anteo's technology and covalently conjugated magnetic particles.

All materials and tests were performed in DCN's laboratories using magnetic particles conjugated with anti-hCG antibody using Anteo's protocol and DCN's standard covalent conjugation procedure. hCG concentrations from 0 to 250 mIU/ml were prepared in 1%BSA, 0.1%Tw-20 1xPBS and in negative pooled human urine. Tests were performed using 100ul of sample and test strips with the covalently conjugated and Anteo's conjugated particles dried onto a conjugate pad. Five replicates for each concentration were tested and results were recorded 15 minutes after the addition of the sample using DCN's visual grading scale and an ESE Quant (Qiagen Lake Constance, Germany) reflectance reader.

The amount of antibody conjugated to the different particles and the amount of particles used per test were optimised to produce the highest sensitivity. Anteo's conjugate was tested with an antibody loading of 25ug of anti-hCG antibody per mg of particles at 0.05% solids. The covalently conjugated particle was tested with an antibody loading of 50ug of anti-hCG per mg particles at 0.05% solids.

Results

The results for the visual grades and the values recorded by the reflectance reader, including standard deviation and %CV are shown in *Table 1* for each of the two tests. The limit of detection for the Anteo assay was ~10 mIU/ml hCG in buffer and ~25 mIU/ml in urine for the visual and reader based results. The limit of detection for the covalently conjugated hCG assay was ~50 mIU/mL in buffer and ~100 mIU/mL in urine. As a reference the limit of the detection for the optimised colloidal gold assay is at ~2.5 mIU/mL in buffer and ~25 mIU/mL in urine.

NOTE: A visual grade of 2 or higher at the test line is considered positive, while the cut-off for the reader is at 30mV.



hCG Concentration (mIU/mL) in Buffer	Anteo Mix&Go Magnetic Particle				Covalently Conjugated Magnetic Particle			
	Visual Grade	Average TL (mv)	Stdev	%CV	Visual Grade	Average TL (mv)	Stdev	%CV
250	9.0	486.9	16.3	3%	5.4	163.5	25.8	16%
100	7.0	314.0	11.3	4%	4.0	108.0	12.3	11%
50	6.0	174.8	8.2	5%	3.0	62.8	6.0	10%
25	4.0	90.9	7.7	8%	2.6	59.5	12.8	21%
10	2.0	36.8	5.1	14%	2.2	44.7	6.2	14%
5	0.0	0.0	0.0	NA	1.8	34.3	11.4	33%
0	0.0	0.0	0.0	NA	1.0	6.5	12.4	189%

Table 1: Visual and Reader results of the Anteo's technology and covalently conjugated magnetic particle based hCG assays in buffer.

Magnetic Particle Conjugation Evaluation hCG spiked in Buffer Dose Response Curve Reflectance Reader (Average of N=5)

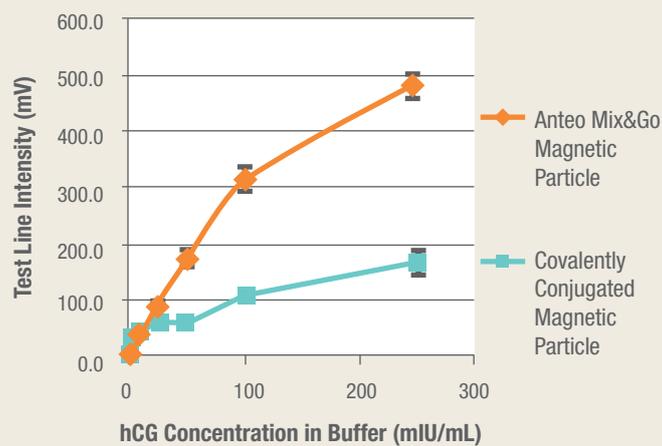


Figure 1: Graph of the results from the Anteo and covalently conjugated magnetic particle hCG assays in buffer measured by a Qiagen reader.

Figures 3 and 4: Pictures of lateral flow strips run with the Anteo and covalently conjugated magnetic particles with hCG diluted in buffer.



Figure 3: Anteo magnetic particle test strips run with hCG diluted in buffer

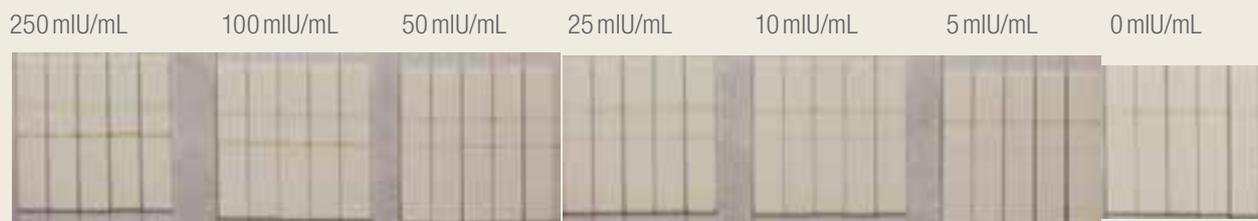
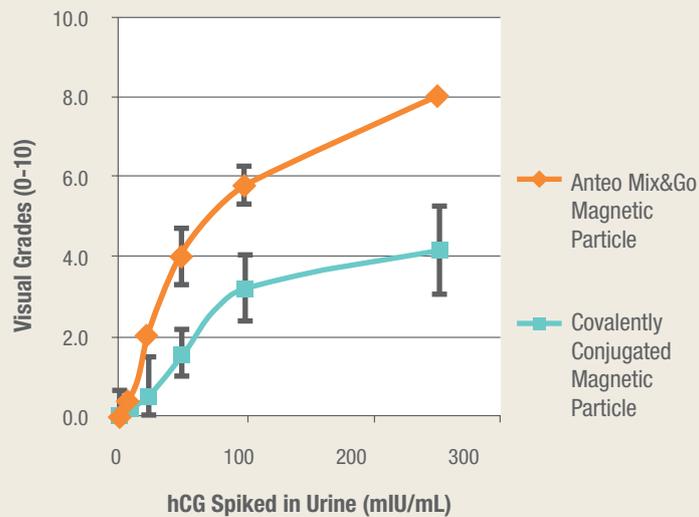


Figure 4: Covalently conjugated magnetic particle test strips run with hCG diluted in buffer.

hCG Concentration (mIU/mL) in Urine	Anteo Mix&Go Magnetic Particle				Covalently Conjugated Magnetic Particle			
	Visual Grade	Reader Average TL (mv)	Stdev	%CV	Visual Grade	Reader Average TL (mv)	Stdev	%CV
250	8.0	378.6	32.0	8%	4.2	86.9	35.2	40%
100	5.8	174.9	26.6	15%	3.2	43.7	29.4	67%
50	4.0	79.8	26.2	33%	1.6	9.0	20.1	224%
25	2.0	10.0	16.0	160%	0.6	7.2	16.0	224%
10	0.2	6.4	14.4	224%	0.2	0.0	0.0	NA
5	0.0	0.0	0.0	NA	0.2	7.8	17.4	224%
0	0.0	0.0	0.0	NA	0.0	0.0	0.0	NA

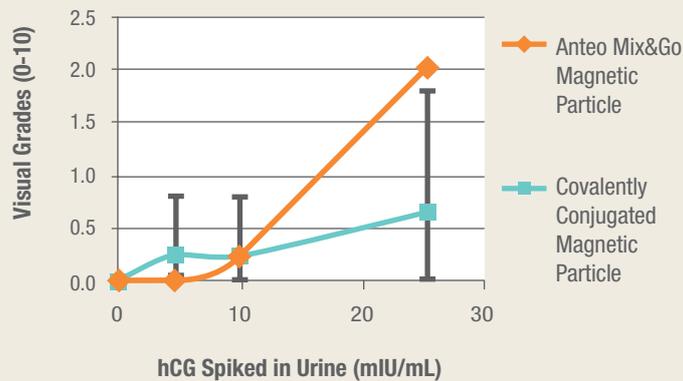
Table 2: Visual and reader results of the Anteo and covalently conjugated magnetic particle hCG assays with urine.

**Magnetic Particle Conjugation Evaluation
hCG Spiked in Urine Dose Response Curve
Visual Grades (Average of N=5)**



Figures 5 and 6: Graphs of the reader results from the Anteo and covalently conjugated magnetic particle hCG assays in urine.

**Magnetic Particle Conjugation Evaluation
hCG Spiked in Urine Dose Response Curve
Visual Grades (Average of N=5; <25mIU/mL)**



Figures 7 and 8: Pictures of the Anteo and covalently conjugated magnetic particle hCG assays run with hCG diluted in urine.



Figure 7: Anteo magnetic particle test strips run with hCG diluted with urine.



Figure 8: Covalently conjugated magnetic particle test strips run with hCG diluted with urine.

Conclusions

The direct comparison of the two tests show:

The Anteo technology based hCG assay is 5 times more sensitive than the covalently conjugated magnetic particle based test using the same critical reagents. The estimated limit of detection for the Anteo assay is ~10 mIU/mL of hCG in buffer and ~25 mIU/mL of hCG in urine while the covalently conjugated hCG assay has limits of detection of ~50 and 100 mIU/ml of hCG in buffer and urine respectively. The hCG assay using the Anteo particles used half the amount of antibody to achieve better sensitivity than the covalently conjugated assay.

The variability of the Anteo technology based hCG assay is lower than the covalently conjugated hCG assay. There is also less non-specific binding with the Anteo particles.

The Anteo technology based magnetic particle hCG lateral flow assay produced a comparable limit of detection to the optimised colloidal gold reference assay of ~25 mIU/mL in urine. This confirms that particles conjugated with the Anteo Activation Reagent can rival traditionally used gold colloids in lateral flow applications and bring with it benefits associated with cost, handling and manufacturability improvements.



Anteo Products Lateral Flow

Anteo's *Lateral Flow Coupling Kit* makes conducting lateral flow tests with magnetic beads easier and more flexible. Magnetic separation removes the need to perform centrifugation and filtration concentration.



Advantages

- An alternative to EDC chemistry the Anteo Kit is easy to use
- Less hazardous chemicals to work with, Anteo's Activation Reagent is water based
- Flexibility: you can bind any antibody subclass to your surface
- Promotes monolayer binding which increases antibody functionality
- Reduces aggregation
- Low background noise

The Anteo Lateral Flow Coupling Kit is launching at AACC 2016. For more information on this kit email sales@anteotech.com

About Anteo Technologies

Anteo Technologies is transforming the way scientists work by providing new, powerful tools developed with their patented nanoscale engineered Activation Reagent. This Reagent significantly advances traditional protein coupling methods, leading to increased assay sensitivity.

Anteo's Activation Reagent is designed by using cross-linking metal ions to enable gentle yet secure multi-point binding of biomolecules to synthetic materials. The Reagent also delivers solutions to the challenges of establishing highly functional biological interfaces between fragile biomolecules, and synthetic and often incompatible hydrophilic/hydrophobic materials such as silica, polystyrene, COC/COP, metals and ceramics.

Water based, Anteo's Activation Reagent is easy to use. Simply pipette the reagent onto your surface to activate. Hands on time for surface activation and coupling is 60 minutes.

Find out more at anteotech.com

References

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