Catalytic Nanoparticles to Enhance the Sensitivity of Lateral Flow Immunoassays

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ABSTRACT

Lateral-flow immunoassays (LFIAs) are a widely used point-of-care technology. They are most familiar as home-pregnancy tests, but are also employed for testing for a wide variety of diseases, biomarkers, and even small molecules. By substituting catalytic particles for the conventional colorimetric labels, we have demonstrated an increase in sensitivity of at least 1000x without placing an undue burden on the user.

Keywords: lateral-flow immunoassays, catalytic label, increased sensitivity

RESULTS

Visually-read, LFIAs typical use colloidal gold or colored latex as the reporter elements for the recognition binding event (Figure 1). While simple to perform and easy to read, the sensitivity of LFIAs is ultimately limited by the extinction coefficients of the reporter elements as enough material must be localized to be visually observed. We have substituted specially-prepared, colloidal palladium for the colorimetric labels and use the palladium nanoparticles as a chemical catalyst to produce an easily-observable, stable, blue dye that localizes at the capture line. We term our test system cLFIA™ for catalytic LFIAs. Importantly, the catalyst is heat stable, functions at room temperature, under physical conditions, and results in up to a 500-fold increase in visual sensitivity with only five additional minutes of development (Figure 2).

Although blue is an optimum, visual color for most biological assays, the dye chemistry is derived from color photography and hair dying products (Figure 3) so that different colors can be produced, spanning the visual spectrum. Some versions of the catalyst have on their surfaces functionalities that covalently bind amines such as the lysines on proteins for facile conjugation. More recent results have optimized the preparation of the catalyst and labeling of proteins even further to a one-step process in the presence of the antibody partner. Removal of excess reagents can be accomplished by simple centrifugation.

Different enhancements and kinetic curves have been observed for different Pd nanoparticle preparations. We have developed a characterization system using an ELISA plate reader (Figure 4A & B). It appears that Pd particles <10 nm give best enhancement, but we need to better characterize the different particles and relate that to catalytic activity. Although many of 100s of catalytic preparations that we have tested behave very similarly, there are slight differences in the kinetics which depend on reagent and Pd particle concentrations, leaving room to optimize LFIA performance for certain applications. Currently, 3-5 min development is enough to see signal over background, which is easily resolvable even at very low amounts of catalyst.

Quantitation can be accomplished by scanning the strip using a commercial flat-bed scanner and processing the data using Image J. With this method, increases in sensitivity 1000 fold over a similar commercial assay have been established (Figure 5).

The catalytic chemistry requires that three chemicals react with the palladium nanoparticle label. We are working to design an assay that incorporates all chemical elements onto the LFIA test strip thereby requiring no additional steps to be performed by the user. An example of the completed assay is shown in Figure 6.

Being more sensitive, our cLFIA™ should greatly expand the applicability of LFIAs, which are the utmost fieldable and user-friendly assays presently available, especially in underserved, resource-poor countries.

REFERENCES

David A. Kidwell, “Catalytic Particles for Increased Sensitivity in Lateral Flow Immunoassays”, February 22, 2018, 20180052153 A1

D.A. Kidwell, E. Wei, J. Wei, and S.P. Mulvaney, "Increasing Lateral Flow Immunoassay Sensitivity Using Catalytic Particles", poster presentation at Biosensors 2018 - 28th Anniversary World Congress on Biosensors, Miami, FL, June 12-15, 2018.

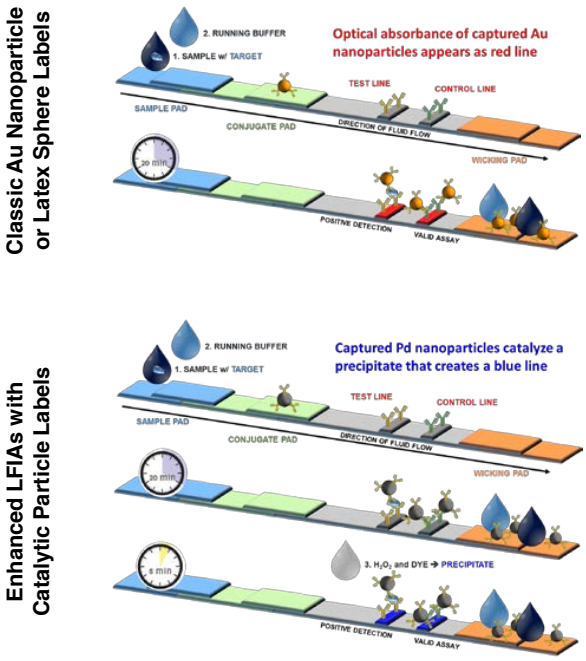

Figure 1 – Gold vs. Palladium labeled LFIAs. Conventional LFIAs use either colloidal gold or dyed particles as the label and their sensitivity is limited by the absorbance of the particles. Our cLFIA™ substitutes catalytic particles for the colorimetric label and uses their catalytic power to produce a visual image. Other types of signals such as chemiluminescence and fluorescence have also been demonstrated and are being explored. However, these alternative signals must be instrumentally read, complicating fielding the assay especially in resource-poor countries.

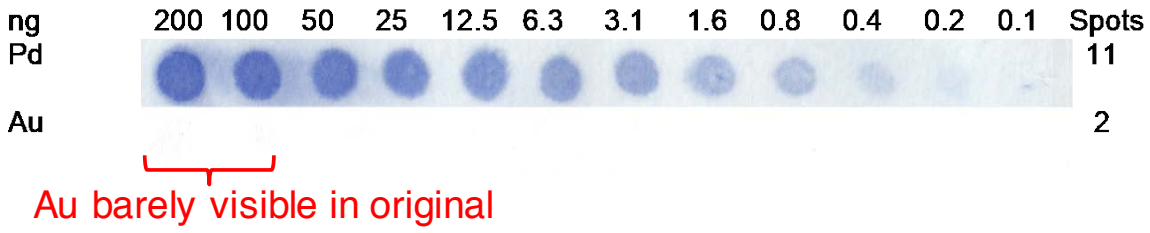

Figure 2 – Signal enhance of catalytic palladium over colorimetric gold labels. Typical LFIAs use 1-2 μg of gold label. Our system obtains a comparable line intensity with only 50 ng of palladium, reducing cost, and increasing sensitivity

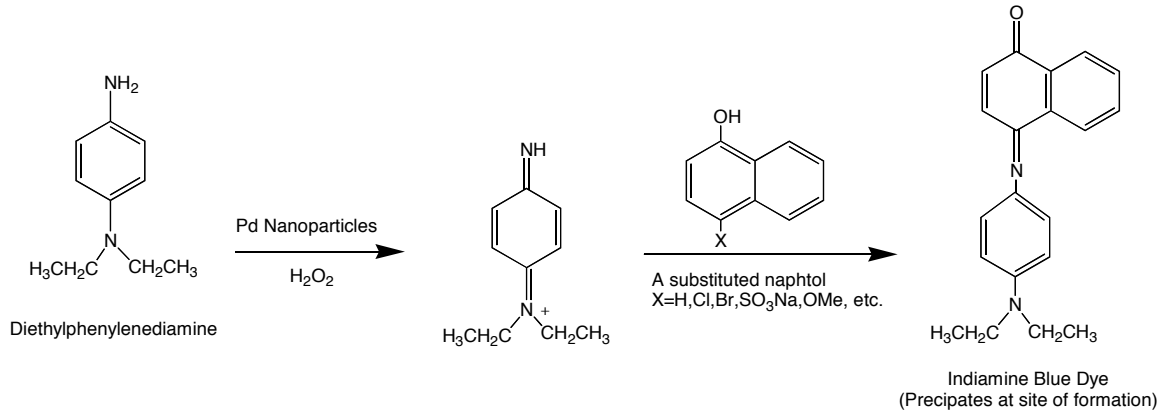

Figure 3 – Basis of the dye chemistry. The catalytic chemistry requires that a napthol, diamine, and hydrogen peroxide react at the Pd nanoparticle to produce the precipitating dye. Fortunately this chemistry it quite flexible. A number of substituted napthols can be used to generate the same indiamine blue dye to optimize cost, solubility, and reaction rate. Substitutions on the phenylenediamine vary the shade of blue and reagent stability. Using phenols in place of a napthol or substitution of an amino phenol for the diamine produces a red/brown color.

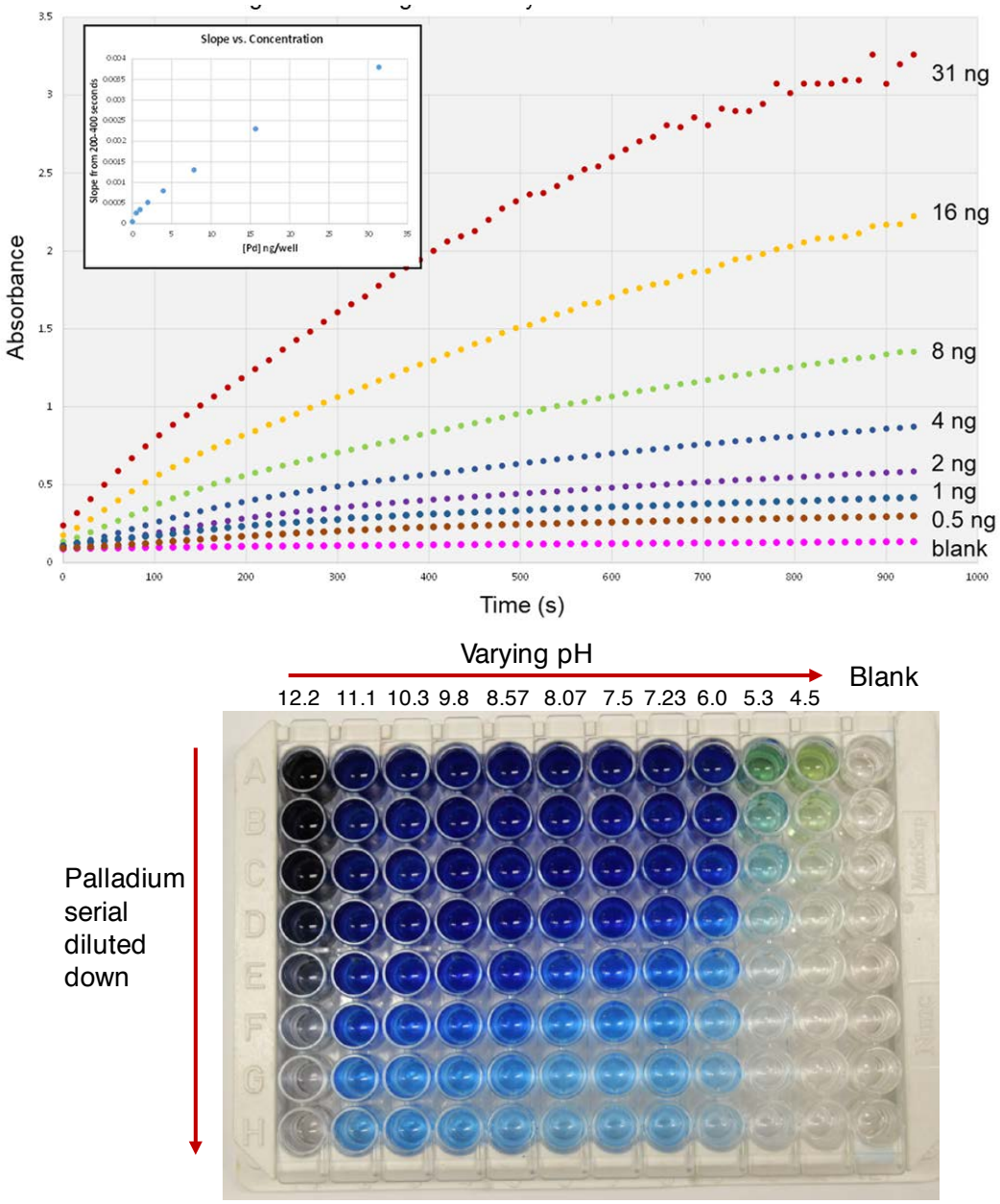

Figure 4 - A) Kinetics in an ELISA plate with indicated amount of Pd. Insert plots slope from 200-400s at each concentration showing a linear response with concentration of the catalyst at a given time. Having a linear response to the label greatly increases the flexibility of the assay. **(B) Catalytic action vs. pH.** The dye system has a wide pH operational range with pH of 8-11 being optimal.

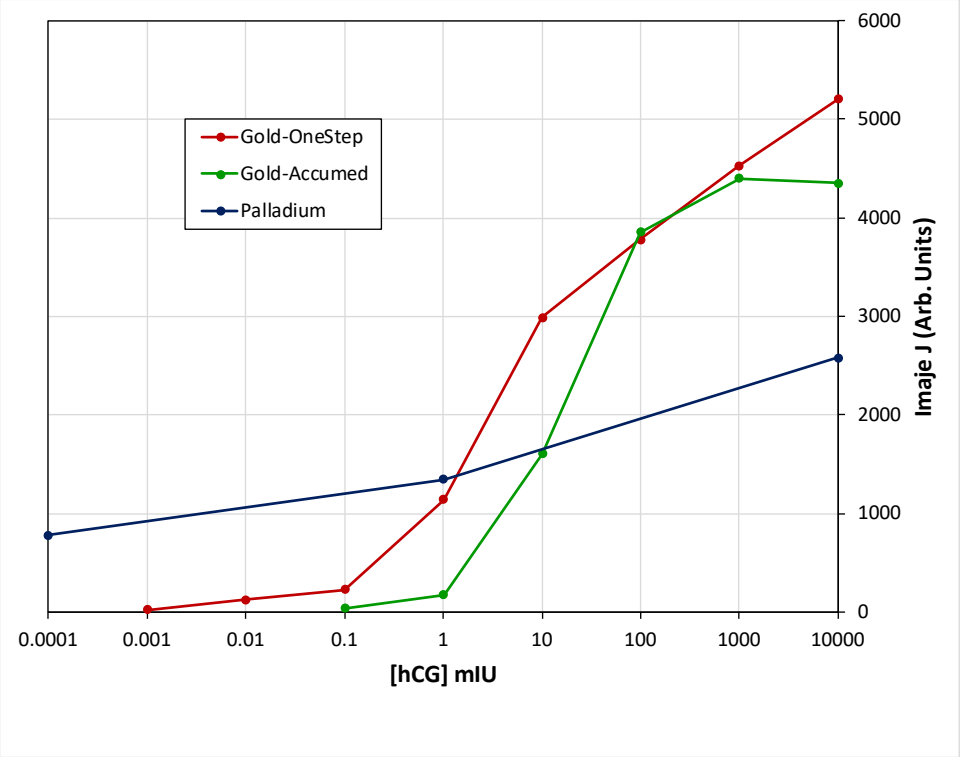

Figure 5 – Comparison of our cLFIA™ assay with commercial hCG assays. We redesigned a home pregnancy test with COTs antibodies using either the commercial One Step or Accumed home pregnancy tests as a comparison. hCG was spiked into buffer and applied to the test. Tests were read after 10 min development by scanning with a flat-bed scanner and digitizing the strip using Image J (digitization increases the sensitivity). The plot above is Image J density units vs. milli-international units of hCG. For visually read strips, typical commercial sensitivities are about 10 mIUs whereas the cLFIA™ strategy shows a ~1000x enhancement in sensitivity in this example.

Figure 6 - Example of a cLFIA™. In this model system, goat anti-mouse antibodies conjugated with Pd nanoparticles were captured at lines (marked C & 2) of mouse IgG antibodies immobilized on a nitrocellulose strip. No capture to a rabbit IgG antibody line (marked 1) was observed. The completed assay was developed with the lines observable in less than one minute. The blue dye can be stable for months although the color density appears different in a wet vs. dry state.