

An immunoassay using antibody-gold nanoparticle conjugate, silver enhancement and flatbed scanner

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Abstract This study reports a convenient immunoassay using antibody-gold nanoparticle (Ab-AuNP) conjugate as a reporter molecule and a flatbed scanner for the optical scanning and measuring of the immuno-reaction signal. The silver enhancement reaction, a signal amplification method in which silver ions are reduced to silver metal, is introduced to magnify the detection signal. The whole framework of the study is based on (1) the direct immunoassay (two-layer format) in which the antigen is directly immobilized on the chip, and (2) the sandwich immunoassay [three-layer format, process similar to enzyme-linked immunosorbent assay (ELISA)], which contains a primary antibody, a secondary antibody (antibody-nanoparticle conjugate) and antigens. The experimental data show that the micro-scale silver precipitation phenomenon is catalyzed by Ab-AuNP conjugates. This reaction can be observed by the naked eye, even at an IgG-AuNPs concentration of 5 pM. The relationships between sample concentration and detection signal are discussed, and the detection limit (sandwich assay) for the sample antigen is

1 ng/mL. Using a flatbed scanner, Ab-AuNP conjugates and a silver enhancement reaction, a new immunoassay is constructed.

Keywords Immunoassay · Silver enhancement · ELISA · Optical · Gold nanoparticle

1 Introduction

Immunoassay plays an important role in clinical observation or serum diagnosis, and is well known (Hage 1999; Hirsch et al. 2003; Nichkova et al. 2005). Fluorescent dyes (Hildebrandt et al. 2005), enzymes (Dill et al. 2006) and nanoparticles (Dequaire et al. 2000) are commonly used as materials for protein labeling in an immunoassay. In recent studies of immunoassay, fluorescent materials (fluorophores or luorochromes) have become the main labels for generating fluorescent signals, which can be detected through various optical or electrochemical methods (Kangatharalingam and Essenberg 2000; Ko et al. 2003). In addition, nanoparticles are being used to enhance the optical detection signal and reduce the background interference (Schneider et al 2000; Lochner et al. 2003; Matveeva et al. 2004; Li et al. 2006; Chumbimuni-Torres et al. 2006; Peng et al. 2007).

The immunogold labeling technique is commonly used for nanoparticle marking (Hayat 1989; Das et al. 2006; Zhang et al. 2007). Due to the high electron density of nanoparticles, the colloidal gold-labeled proteins show up as dark brown under the electron microscope, resulting in more distinct images for labeling. This image response can be further enhanced through silver precipitation on the nanoparticles, called immunogold silver staining (IGSS) (Holgate et al. 1983; Hayat 1995; Peter 1996). In addition,

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IGSS can highly increase the ability of the traditional electron and optical microscopes for analysis and observation. Generally speaking, the applications of IGSS of human tissues, such as eukaryotic tissues and malignant tissues, were mostly found to mark cell organelles or to detect protein immunoreactions (Springall et al. 1984; Scopsi and Larsson 1985; Jackson et al. 1989; Wang et al. 2001; Chu et al. 2005). The binding of antibody (Ab) and antigen can be detected by many techniques. However, only a few are suitable for widespread use due to the bulky and expensive instruments required by many of these detection techniques.

Recently, Mirkin et al. demonstrated that nanoparticles can be labeled to DNA molecules to produce a detection signal, and when combined with the silver enhancement solution, the silver signal becomes more strengthened for better sensitivity (Taton et al. 2000; Park et al. 2002; Hurst et al. 2006). The principle here is that the reduction response (from ionic silver to metal silver) can be catalyzed by gold nanoparticles (AuNPs), causing silver precipitation on the surface of the AuNPs. The silver covering on the AuNPs then serve as a biological transducer, transforming the phenomenon of nano-scale biomoleculars to detectable signals.

In the study, we used two model immunoassay formats (direct and sandwich immunoassay), as shown in Fig. 1. For the direct immunoassay, the test antigens were immobilized on the glass slides, and the AuNP-labeled rabbit IgG were then bound to the antigens, resulting in the

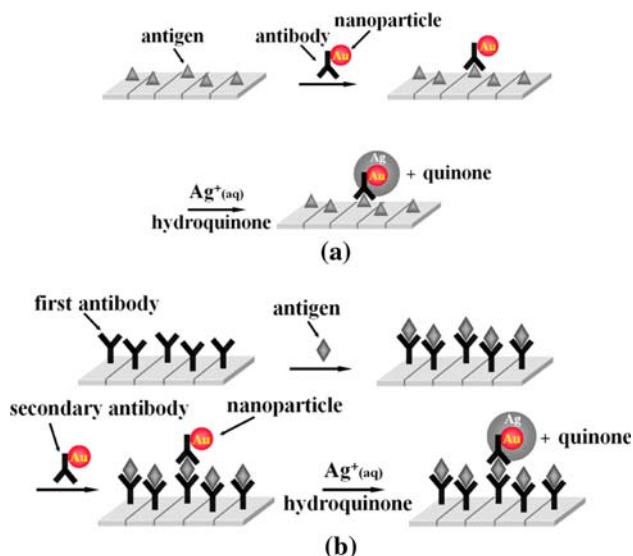


Fig. 1 Schematic drawings of the signal amplification method for immunoassay in which silver ions are reduced by hydroquinone to bulk silver metals, which results in the silver cover on the AuNPs surfaces. **a** The direct format assay for evaluating the silver precipitation effects. **b** The sandwich format assay for performing the final ELISA-like immunoassay

formation of a two-layer complex. For the proposed sandwich immunoassay (ELISA-like), the first antibodies were immobilized on the glass slides, and the test antigens were then bound to the first antibodies. When the AuNP-labeled rabbit IgG were added to the chip, they would bind to the test antigens which were already bound to the first antibodies, resulting in the formation of a sandwich complex.

Based on the superior performance of the DNA assay proposed by Mirkin group, we utilized it in this work for immunoassay. In this study, our strategy is based on using the AuNPs probe coupled with silver enhancement for the immunoassay, and using a conventional desktop scanner for the signal measurement. Protein A (extracted from *Staphylococcus aureus*) and Immunoglobulin G (IgG, obtained from human or goat) were employed as the model immunoassay for their high specificity and convenience. This study illustrates the use of AuNPs, followed by AuNPs catalyzed silver enhancement, to detect Protein A and IgG interactions. This study first shows that AuNPs catalyze silver precipitation and they go on to show that protein interactions on surfaces can be enhanced by precipitating Ag on AuNPs that are already specifically bound to the surface. IgG-AuNPs conjugates were used as the detection probe to catalyze the reduction of silver ion to silver metal, resulting in a particle-size enlargement and color differentiation. The optical signal produced from the binding of nanoparticle-labeled proteins could then be observed by the naked eye or by an optical instrument while the silver enhancement solution was introduced to cause the silver amplification effect. The characteristics of silver precipitation catalyzed by AuNPs were produced to analyze the effects of IgG-AuNPs without any binding reactions, which could be observed by the naked eye or measured quantitatively by a silver ion-meter. When all the proper conditions were established on the glass slide, a desktop scanner was used to capture the optical signal (digital images) which was then processed in a personal computer for data analysis. The concentration effects and the detection limit were then examined for silver precipitation immunoassay.

2 Materials and methods

2.1 Reagents

Hydrogen tetrachloroaurate(III), silver enhancement solution, human serum IgG, protein A, phosphate-buffered saline (PBS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, and sodium citrate (dihydrate, granular) was obtained from J. T. Baker. Sodium borohydride powder (98+%) was obtained from ACROS.

2.2 Labeling of IgG to gold nanoparticles (AuNPs)

Five nanometer AuNPs were prepared by a modified citrate-reduction process (Grabar et al. 1995). An aqueous tetrachloroaurate solution (1.0 mM, 100 mL) was cooled at near 0°C in an ice bath, and then trisodium citrate (0.17 M, 10 mL) was added to the solution. NaBH₄ solution (0.011 M, 20 mL) was then added dropwise to the Au(III)-citrate solution. The color change of the solution from blue to red indicated the formation of monodispersed spherical nanoparticles (~5 nm). The surfaces of the AuNPs, in a solution at pH 6.2, covered with citrate anions, indicated a negative charge. The value of *pI* of IgG is close to 7.0, and therefore, in a pH 6.2 solution IgG should bear a net positive charge. Consequently, when the IgG solution was added to the AuNPs solution, the IgG became adsorbed onto the surfaces of the AuNPs through ionic interactions. The mixture of IgG (25 µg/mL, 0.4 mL) and gold nanoparticles (4 mL) was incubated at room temperature for 60 min to generate the IgG-AuNPs conjugates and centrifuged at 21,000 rpm (23,100 g) for 60 min, and the IgG-AuNPs conjugates was then observed in the bottom of the mixed solution. We discarded the supernatant and examined conjugation using UV-visible absorption spectroscopy.

2.3 Coating slides with antigen or antibody

First, the slides were rinsed with deionized water (DI water) and dried with nitrogen gas and then covered with polydimethylsiloxane (PDMS) thin film in which holes were punched (regular-size hole puncher) to form wells on the surface of the slides (The dimension of slides is 76 × 22 mm²). The size of the wells corresponds to the well size generally used in 96-well plates (7 mm in diameter). A coating solution of IgG (10–30 µg/mL of IgG dissolved in PBS, 50 mM, pH 7.4) was added, and the slides were incubated for 3 h at room temperature in a humid chamber (37°C, 5% CO₂ and pH = 7.2, REVCO-ULYIMA, in USA). Then, IgG covalently bound to the surface of slide/the bottom of well (25 µL/well). They were rinsed with DI water and washing solution (0.05% Tween 20 in DI water). Then, blocking was performed by adding blocking solution (1% BSA, and 0.05% Tween-20 in 50 mM PBS buffer, pH 7.4, 35 µL per well) and incubating overnight at 37°C in a humid chamber.

2.4 End-point binding

AuNPs-labeled rabbit IgG (diluted to 20 µg/mL with PBS buffer, 50 mM, pH 7.4) was added to the direct format slide (coated with protein A) or the sandwich format slide [coated with IgG and antigen (protein A)] (25 µL/well),

and the slides were then incubated at 37°C in a humid chamber for 1–2 h. Slides were then rinsed with DI water and washing solution (0.05% Tween-20 in water), coated with blocking buffer, and stored at 37°C before measurement.

2.5 Instrumentation and scanometric image signal caption

The detection system and procedure are shown in Fig. 2. The desktop scanner (Scanjet 3400C) was purchased from Hewlett-Packard, USA. The gray level depth was set to 8 bits, so there were 256 values for each gray level variation. The image caption was patterned as a circular area with 6,948 pixels to fit the reaction well on the slide. In addition, absorption spectra of AuNPs and AuNPs-labeled Abs in solution were measured using a Hewlett-Packard Agilent 8453 spectrophotometer. In the signal amplification, 30 µL of the silver enhancement solution was added to form the image signal captured after drying. For silver precipitation kinetics, the fix solution (DI water) was added to the well and the grayscale signal was immediately scanned and analyzed by means of a PC. Results were expressed as the means ± standard deviation (*n* = 3). The statistical difference was assumed to be significant when *P* < 0.05 by the two-side Student *t*-test.

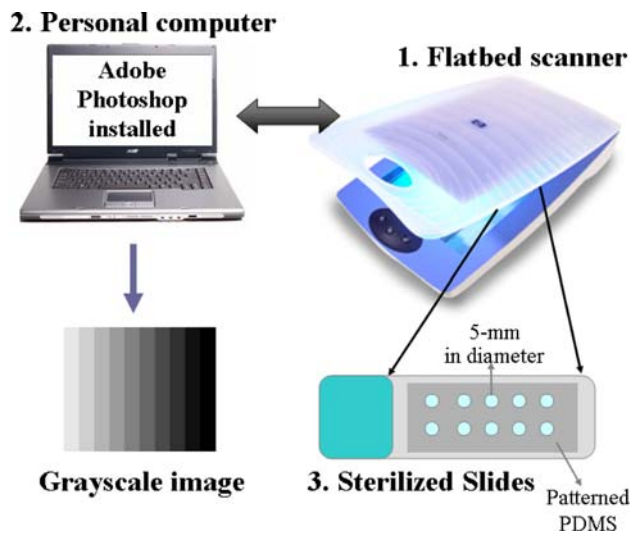


Fig. 2 Detection instrumentation for silver-precipitation immunoassay. The reaction well for the assay is patterned with a circular area using PDMS as the material. After the immuno-reaction and silver enhancement are completed on the glass slide, the detection signal scanned from the flatbed scanner is transferred to a personal computer with Adobe Photoshop® installed. The signal can then be processed to form the eventual gray level data

3 Results and discussion

3.1 Model immunoassay

A model antigen, rabbit IgG, was immobilized on the surfaces (cleaned slide glass surface), and the specific or nonspecific labeled anti-rabbit Ab was allowed to bind to the antigen. For end-point binding measurements, plates were incubated with Ab for approximately 1–2 h to complete the binding reaction. For the direct immunoassay, the test antigens were immobilized on the glass slides, and the AuNP-labeled rabbit IgG were then bound to the antigens, resulting in the formation of a two-layer complex. In this way, we could analyze the relationships between concentrations of test antigen and variation of the impedance to achieve a qualitative analysis.

The proposed sandwich immunoassay (ELISA-like) is a good way to provide qualitative and quantitative analyses. The first antibodies were immobilized on the glass slides, and the test antigens were then bound to the first antibodies. Excess unbound antigens were removed by washing with doubly distilled water. When the AuNP-labeled rabbit IgG were added to the chip, they would bind to the test antigens which were already bound to the first antibodies, resulting in the formation of a sandwich complex.

3.2 IgG-AuNPs conjugates

The solid line represents the absorption of the neat 5 nm AuNPs solution, and the dotted line represents the UV absorption of the IgG-AuNPs solution obtained after centrifugation. The formation of IgG-AuNPs conjugates were evidenced by a red shift in the peak absorption from 517 nm (5 nm AuNP) to 524 nm, indicating a successful conjugation between the 5 nm AuNPs and the IgG molecules (Hayat 1989; Zhou et al. 2002).

3.3 Silver precipitation catalyzed by AuNPs

In order to test the applicability of the present assay format, IgG-AuNPs conjugates were employed as a model. Results show that the IgG-AuNPs conjugates reduced silver ion to silver metal in the presence of a silver enhancer solution. It is evident that IgG-AuNPs conjugates, like AuNPs, retain the ability to reduce silver ions. Figure 3 shows the real-time color change of the IgG-AuNPs in the presence of the silver enhancer solution. The color change could be observed with the naked eyes within a period from 5 s to 6 min at an IgG-AuNPs concentration of 5 pM. For the highest concentration of IgG-AuNPs solution, the gray level values reached the saturation point promptly after about 3 min, indicating that all silver ions to be reduced to the silver metal by catalysis (Fig. 4). When the

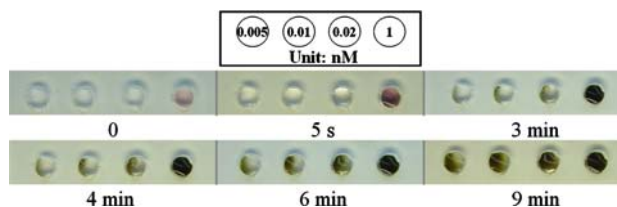


Fig. 3 Real-time color change of IgG-AuNPs conjugates immersed in the silver enhancer solution. The concentrations of the four wells are 0.005, 0.01, 0.02, and 1 nM (from left to right), respectively. The color change is from *colorless* (0.005–0.020 nM) or to *light gray* and *black*. This indicates that IgG-AuNPs conjugates can successfully catalyze the reduction of silver ion to silver metal

concentrations were lowered to 0.01 nM, the change profiles of the gray level values were similar to each other, indicating that the saturation concentration had been reached.

When using an alternative method with a silver ion-meter, the reduction of silver ion to silver metal was assumed to occur on the AuNPs surface of the IgG-AuNPs conjugates. Figure 4b shows that the consumption of the

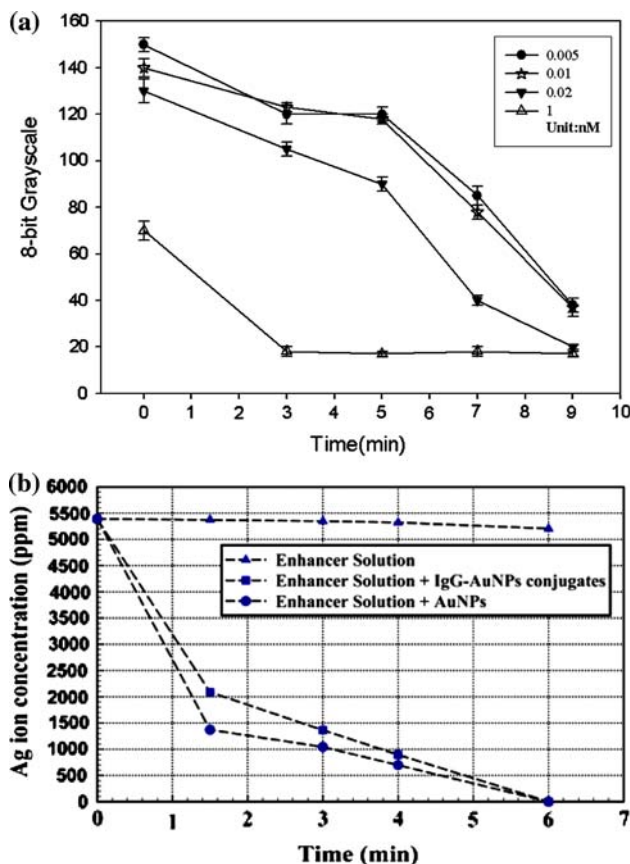


Fig. 4 a Dynamic 8-bit gray-scale value change due to silver precipitation (the silver enhancement solution concentrations included 0.005, 0.01, 0.02, 1 nM) and b Dynamic silver-ion consumption due to IgG-AuNPs conjugates

silver ions in the reaction solution can help reversely evaluate the quantity of the bulk silver that has been reduced by the Quinone⁺² resultant trend conforming to the earlier consumption. In addition, IgG-AuNPs conjugates show a similar ability to reduce silver ion as the neat AuNPs. In the experiments, we find that IgG-AuNPs conjugates concentrations as low as picomolar range could be detected. Obviously, the IgG-AuNPs is an ideal label and only a small sample volume is needed.

3.4 Direct immunoassay

The direct model is an alternative method for inspecting the relationships between the coated antigen and the gray signal generated. Under the same conditions, the coated antigen amount was assumed to be in proportion to the concentration of the coating solution; i.e. the higher the coated sample antigen was, the lower the gray value. The binding solution of IgG-AuNPs conjugates was used at the same concentration. The concentrations of the sample antigen (protein A) were 10, 1, 10⁻¹, 10⁻² and 10⁻³ μg/mL. The concentration of 10⁻⁴ μg/mL was too low for coating the slide. Each experiment was performed with a repetition of three times to ensure the accuracy of the results. Two models of negative control were used to confirm the accuracy of experiments, one with nonspecific BSA (2%, in 50 mM PBS buffer) as negative control (NC) to replace Protein A (Fig. 5a). Figure 6a shows that the change of gray depths can be directly observed by the naked eye. For obtaining more precise differentiation of sample concentrations, the gray values were measured and are listed in Fig. 7a. The antigen concentration was mapped, respectively, to each gray level value detected, which provides the relation between the sample concentrations and the detection signals. The gray levels of 10⁻³ and 10⁻² μg/mL concentrations were of

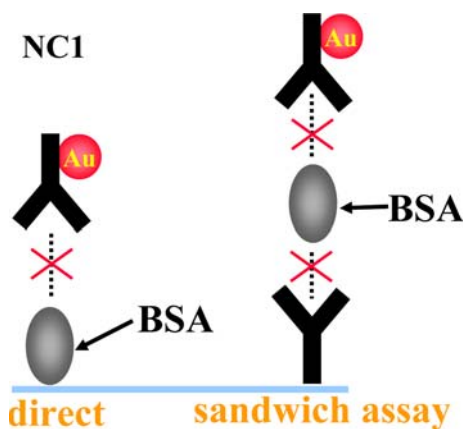


Fig. 5 The model of negative control was used in direct and sandwich assay experiment, NC was used nonspecific BSA (2%, in 50 mM PBS buffer) as control to replace Protein A

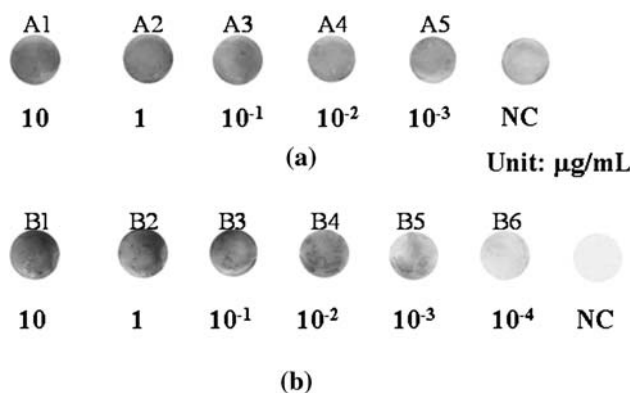


Fig. 6 The corresponding gray level images from different sample antigen concentrations (10 ~ 10⁻⁴ μg/mL): **a** protein A concentrations (10 ~ 10⁻³ μg/mL) in the direct format were corresponded to A1–A5, and **b** protein A concentrations (10 ~ 10⁻⁴ μg/mL) in the sandwich format were corresponded to B1–B6. The difference among protein A concentrations and negative control can be easily observed by the naked eye

similar values, indicating that the detection limit for the direct format is 10⁻² μg/mL.

3.5 Sandwich immunoassay

The proposed sandwich model is similar to the common sandwich of the ELISA, different only in the materials of protein labeling (enzymes vs. AuNPs) and signal amplification process. The proposed model can provide qualitative and quantitative analyses and more sensitive results just like ELISA. IgGs (1st Ab) were coated on the slide as the first layer for binding sample antigens (protein A). Then various concentrations of protein A were immobilized as the second layer by the IgG coating layer. Finally, the corresponding IgG-AuNPs conjugates were added to produce the optical detection signal. Different from the direct format, the sandwich format was used for lower sample concentrations due to the first IgG coating layer. The strong affinity between IgGs and protein A could offer more immobilization for lower concentrations of sample protein A. Hence, the concentrations of the sample antigen (protein A) in the sandwich format were 10, 1, 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ μg/mL. Each experiment was also performed with a three times repetition so as to ensure the accuracy of the results. Figure 6b shows that the change of gray depths can also be observed directly by the naked eye. The gray values were also measured and are listed in Fig. 7b. The antigen concentration was, respectively, mapped to each gray level value detected (from 10 to 10⁻³ μg/mL), which provides the relation between the sample concentrations and the detection signals. The gray level value of 10⁻⁴ μg/mL concentration approaches the gray value of the control experiments (control: 211.65, 10⁻⁴ μg/mL: 209.18), indicating that the detection limit for sandwich format is 10⁻³ μg/mL.

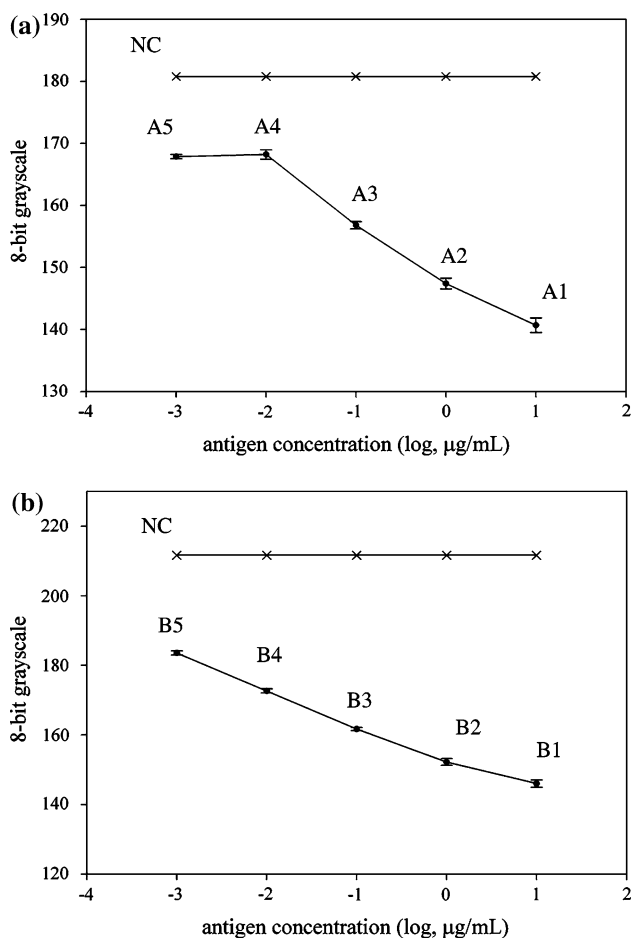


Fig. 7 Graph of 8-bit gray-scale values as a function of reaction time. The dynamic ranges of different concentrations can be determined from the gray scale. Each gray-scale value of concentrations was corresponded to **a** (symbol A1–A5) and **b** (symbol B1–B6) independently

4 Conclusion

This study proposed a novel immunoassay based on a flatbed optical scanner to measure the immuno-reaction signal, with AuNPs as a label of antigen or antibody and as a catalyst for silver precipitation, and with the silver enhancement reaction to magnify the detection signal. A simple detection of staining color was correlated to the analyte concentration for quantitative and qualitative analyses. The results are an interesting and attractive method for immunoassay systems in terms of simplicity, sensitivity, and cost. This method has several advantages: (1) the implementation of an assay is easy, (2) only a small amount of reagent is required (as little as 25 µL of sample protein per well detection), (3) it is fast (optical detection within 15–30 min), and (4) convenient and low-cost detection (an ordinary desktop scanner). This method has many potential usages for protein microarray research and clinical diagnosis.

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References

- Chu X, Fu X, Chen K, Shen GL, Yu RQ (2005) An electrochemical stripping metalloimmunoassay based on silver-enhanced gold nanoparticle label. *Biosens Bioelectron* 20:1805–1812
- Chumbimuni-Torres KY, Dai Z, Rubinova N, Xiang Y, Pretsch E, Wang J, Bakker E (2006) Potentiometric biosensing of proteins with ultrasensitive ion-selective microelectrodes and nanoparticle labels. *J Am Chem Soc* 128:13676–13677
- Das J, Aziz MA, Yang H, (2006) A nanocatalyst-based assay for proteins: DNA-free ultrasensitive electrochemical detection using catalytic reduction of p-Nitrophenol by gold-nanoparticle labels. *J Am Chem Soc* 128:16022–16023
- Dequaire M, Degrand C, Limoges B (2000) An electrochemical metalloimmunoassay based on a colloidal gold label. *Anal Chem* 72:5521–5528
- Dill K, Ghindilis A, Schwarzkopf K (2006) Multiplexed analyte and oligonucleotide detection on microarrays using several redox enzymes in conjunction with electrochemical detection. *Lab Chip* 6:1052–1055
- Grabar KC, Freeman RG, Hommer MB, Natan MJ (1995) Preparation and characterization of Au colloid monolayers. *Anal Chem* 67:735–743
- Hage DS (1999) Immunoassays. *Anal Chem* 71:294–304
- Hayat MA (1995) Immunogold-silver staining overview. CRC Press, New York, p 315
- Hayat MA (ed) (1989) Colloidal gold: principles, methods, and applications. Academic Press, New York 1:252–316
- Hayat MA (ed) (1989) Colloidal gold: principles, methods, and applications. Academic Press, New York, vol 1, p 15
- Hildebrandt N, Charbonniere LJ, Beck M, Ziessel RF, Löhmansröben HG (2005) Quantum dots as efficient energy acceptors in a time-resolved fluoroimmunoassay. *Angew Chem Int Ed* 44:7612–7615
- Hirsch LR, Jackson JB, Lee A, Halas NJ, West JL (2003) A whole blood immunoassay using gold nanoshells. *Anal Chem* 75:2377–2381
- Holgate CS, Jackson P, Cowen PN, Bird CC (1983) Immunogold-silver staining: new method of immunostaining with enhanced sensitivity. *J Histochem Cytochem* 31:938–944
- Hurst SJ, Lytton-Jean AKR, Mirkin CA (2006) Maximizing DNA loading on a range of gold nanoparticle sizes. *Anal Chem* 78:8313–8318
- Jackson P, Teasdale J, Cowen PN (1989) Development and validation of a sensitive immunohistochemical oestrogen receptor assay for use on archival breast cancer tissue. *Histochem Cell Biol* 92:149–152
- Kangatharalingam N, Essenberg M (2000) Modified pyrogallol-initiated immunogold-silver enhancement technique applicable to prokaryotes. *J Microbiol Methods* 41:211–217
- Ko JS, Yoon HC, Yang H, Pyo HB, Chung KH, Kim SJ, Kim YT (2003) Polymer-based microfluidic device for immunosensing biochips. *Lab Chip* 3:106–113
- Li ZP, Liu CH, Fan YS, Wang YC, Duan XR (2006) A chemiluminescent metalloimmunoassay based on silver deposition on colloidal gold labels. *Anal Biochem* 359:247–252
- Lochner N, Lobmaier C, Wirth M, Leitner A, Pittner F, Gabor F (2003) Silver nanoparticle enhanced immunoassays: one step

- real time kinetic assay for insulin in serum. *Eur J Pharm Biopharm* 56:469–477
- Matveeva E, Gryczynski Z, Malicka J, Gryczynski I, Lakowicz JR (2004) Metal-enhanced fluorescence immunoassays using total internal reflection and silver island-coated surfaces. *Anal Biochem* 34:303–311
- Nichkova M, Dosev D, Gee SJ, Hammock BD, Kennedy IM (2005) Microarray immunoassay for phenoxybenzoic acid using polymer encapsulated eu:Gd₂O₃ nanoparticles as fluorescent labels. *Anal Chem* 77:6864–6873
- Park SJ, Taton TA, Mirkin CA (2002) Array-based electrical detection of DNA with nanoparticle probes. *Science* 295:1503–1506
- Peng Z, Chen Z, Jiang J, Zhang X, Shen G, Yu R (2007) A novel immunoassay based on the dissociation of immunocomplex and fluorescence quenching by gold nanoparticles. *Anal Chim Acta* 583:40–44
- Peter ML (1996) Immunogold silver staining for light microscopy. *Histochem Cell Biol* 106:9–17
- Schneider BH, Dickinson EL, Vach MD, Hoijer JV, Howard LV (2000) Optical chip immunoassay for hCG in human whole blood. *Biosens Bioelectron* 15:597–604
- Scopsi L, Larsson LI (1985) Increased sensitivity in immunocytochemistry. Effects of double application of antibodies and of silver intensification on immunogold and peroxidase-antiperoxidase staining techniques. *Histochem Cell Biol* 82:321–329
- Springall DR, Hacker GW, Grimelius L, Polak JM (1984) The potential of the immunogold-silver staining method for paraffin sections. *Histochem Cell Biol* 81:603–608
- Taton TA, Mirkin CA, Letsinger RL (2000) Scanometric DNA array detection with nanoparticle probes. *Science* 289:1757–1760
- Wang J, Polsky R, Xu D (2001) Silver-enhanced colloidal gold electrochemical stripping detection of DNA hybridization. *Langmuir* 17:5739–5741
- Zhang SB, Wu ZS, Guo MM, Shen GL, Yu RQ (2007) A novel immunoassay strategy based on combination of chitosan and a gold nanoparticle label. *Talanta* 71:1530–1535
- Zhou Y, Itoh H, Uemura T, Naka K, Chujo Y (2002) Synthesis of novel stable nanometer-sized metal (M = Pd, Au, Pt) colloids protected by a π -conjugated polymer. *Langmuir* 18:277–283