Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 1035–1040

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Barcode lateral flow immunochromatographic strip for prostate acid phosphatase determination

Cheng Fang[a,b,*], Zhencheng Chen[b,**], Lin Li[b], Jinhong Xia[b]

[a] Department of Biomedical Engineering, School of Geosciences and Info-Physics, Central South University, Changsha 410083, China
[b] School of Life and Environment Science, Guilin University of Electronic Technology, Guilin 541004, China

ARTICLE INFO

Article history:
Received 12 April 2011
Received in revised form 2 August 2011
Accepted 4 August 2011
Available online 10 August 2011

Keywords:
Barcode
Immunochromatographic strip
Prostate acid phosphatase
Enzyme linked immunosorbent assay

ABSTRACT

A barcode semiquantitative lateral flow immunochromatographic strip for prostate acid phosphatase (PAP) was developed, in which the monoclonal antibody specific for PAP was labeled to gold nanoparticle and another monoclonal antibody was immobilized on nitrocellulose membrane in the barcode fashion respectively. Based on the stepwise capture of analyte, the system expresses the concentration of PAP in nanogram range as four distinct ladder bars in 30 min, therefore, which could be detected directly by naked eye or image analyzer. Serum PAP from 65 patients was detected with this method and compared with enzyme linked immunosorbent assay (ELISA). There is a good agreement between the methods. Its easily readable result, and also its simplicity and low cost offers an alternative for testing PAP. By incorporating with different specific antibody, the assay can be further extended to detect a variety of analytes with clinical importance.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Prostate cancer is a malignant tumor, the second leading cause of cancer death among older men [1]. Embarrassedly, some men diagnosed with prostate cancer have the side-effects of treatment with little chance of benefit, such as erectile dysfunction and urinary incontinence. Clearly, we need a better way of detecting prostate cancer at its earliest stages without any symptoms.

Two screening tests commonly used to detect prostate cancer in the absence of symptoms are the digital rectal exam (DRE), a manual exam of the prostate area, and the prostate-specific-antigen (PSA) blood test. Neither of them is perfect for prostate cancer. Most men with an elevated PSA level do not have prostate cancer, and some men with prostate cancer have a low PSA level [2]. The DRE also results in many false positives and false negatives. Using both screening methods together will miss fewer cancers (lower sensitivity) but also increases the number of false positives (lower specificity), which can lead to more testing (usually biopsies of the prostate) and possibly result in medical complications [3]. Prostatic acid phosphatase (PAP) was used to monitor and assess progression of prostate cancer until the introduction of prostate specific antigen (PSA). Recent work, suggesting it has a role in prognosticating intermediate and high-risk prostate cancer, has led to renewed interest in this marker [4]. Combined with the PAP test, the diagnostic accuracy of prostate cancer certainly will improve further.

Most of the PAP assays on the market are based on the solid phase enzyme-linked immunosorbent assay (ELISA). It is usually performed only by the clinical laboratory and require quality assurance and stricter personnel requirements. Moreover, it is time-consuming so that result is not instantly available. Therefore, these tests are not ideally suited for the establishment of large screening programmers to be potentially carried out in the urological clinic as well as in homes and other community settings.

Different promising approaches for rapid and decentralized immunoassay include immunosensors and immunostrips. In spite of the high selectivity and sensitivity, immunosensors normally exhibit the lack of stability, the limited reproducibility and the necessity for the addition of external reagents. Up to now, membrane-strip test devices in either lateral flow or flow through immunoassay formats have provided the major alternatives to centralized immunochemical testing because they are rapid, portable, low-cost, and do not require complicated equipments and technical expertise [5]. Most immunostrip tests are based on the use of colloidal gold antibody tracers, which, due to the pink color of the label, may be detected visually, thus providing rapid on-site qualitative information, or semi-quantitative information, by comparison with a color scale card, about the target analyte [6]. Also, the quantitative analytical data can be achieved by measuring color intensity by densitometry [7,8] or measuring the conductivity or electrochemical stripping of colloid gold labels by electrochemical technology [9,10]. However, with regard to the remaining approaches reported,

* Corresponding author at: Department of Biomedical Engineering, School of Geosciences and Info-Physics, Central South University, Changsha 410083, China.
** Corresponding author. Tel.: +86 773 2292232; fax: +86 773 2292233.
E-mail addresses: fangpingchuan@163.com (C. Fang), chenzhcheng@163.com (Z. Chen).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.08.008

they have not been translated into viable commercial device and PAP has been used rather as a model analyte to demonstrate new technology for the development of immunosensors [11].

In this study, a non-instrumental approach has been developed where the color signal is presented in a ladder bar format. The assay principle is based on the stepwise capture of colorimetric tracer labeled antibody–antigen complex by the immobilized antibody on each successive line [12–14]. The number of lines appearing on the strip is directly proportional to the analyte concentration. The readable signal of the test strip offers a cheaper, disposable and more user-friendly assay which is suitable for public users as a quick screening test.

2. Materials and methods

2.1. Chemicals and materials

Matched prostatic acid phosphatase monoclonal antibody (clone M01010921 and clone M01010922, excellent for ELISA and IRMA) and prostatic acid phosphatase antigen (>98% by SDS-PAGE (reduced)), were purchased from Fitzgerald Industries International (Acton, MA, USA). M01010921 was used as the capture antibody matched with M01010922 as the detection antibody. Goat anti-mouse IgG was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Bovine serum albumin (BSA), Tween 20, hydrogen tetrachloroaurate (III) tetrahydrate, trisodium citrate anhydrous and sucrose, were purchased from Aladdin-Reagent (Shanghai, China). All other chemicals were of analytical reagent grade. Unless specified specially, phosphate buffer (hereinafter referred to as PB) was usually 20 mM, pH 7.4 containing 0.1% sodium azide. Deionized water was used throughout the study.

Nitrocellulose membrane (UniSart^{TM} CN 140) was purchased from Sartorius Stedim Biotech GmbH (Goettingen, Germany), plasma separation membrane (Vivid^{TM} GR) from Pall Life Sciences (Ann Arbor, MI, USA), polyester membrane (Grade 7589) from Ahlstrom Filtration L.L.C. (Holly Springs, PA, USA), and cellulose membrane (H-5076), backing support (J-B8) from Shanghai Jiening Biotech Co., Ltd. (Shanghai, China).

Sixty-five human samples from patients were obtained from the Urology Department of the Second Xiangya Hospital of Central South University. PAP-free serum derived from health person and was proved that it almost did not contain the PAP by the human ELISA Kit assay (Diagnostic Automation, Inc., Calabasas, CA). The study protocol was approved by the Clinical Research Ethical Committee of the Faculty of Medicine, the Central South University. The protocol was thoroughly explained to the patients, and signed consent was obtained. The blood samples were collected within 72 h after the patients' arrival at the hospital and were centrifuged at $1000 \times g$ for 15 min to remove the blood cells. The serum aliquots were stored at −80 °C for subsequent analysis.

2.2. Preparation of colloidal gold

Gold colloids were prepared by controlled reduction of gold chloride with sodium citrate using the similar procedure described by Frens [15]. The procedure for preparing colloidal gold with 20 nm diameter was as follows. Firstly, 200 ml of 0.02% hydrogen tetrachloroaurate (III) tetrahydrate was boiled thoroughly for 5 min. Then 7 ml solution of 1% trisodium citrate was added under constant stirring (600 r/min) and was boiled for another 5 min after the color of the solution had changed in less than 2 min. After cooling, deionized water was added to the initial volume. The diameter of the particle was checked with a transmission electron microscope (Hitachi H-7100, Tokyo, Japan) and UV/Vis spectrograph (Shimadzu UV-1201, Kyoto, Japan).

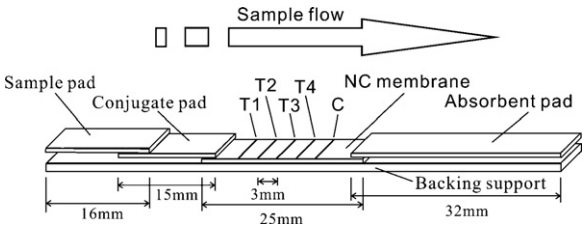

Fig. 1. Schematic of barcode-styled semiquantitative PAP colloidal gold immunostrip showing its main components.

2.3. Conjugation between colloidal gold and antibody

Under gentle stirring in ice bath, anti-PAP monoclonal antibody (M01010922) was added at 5 μg/ml drop by drop to the gold colloidal suspension within 20 min; After overnight incubation at 4 °C, the mixture was centrifuged at 12,000 rpm (centrifugal force $10{,}464 \times g$) at 4 °C for 40 min, and the pellet was resuspended in conjugate storage solution (PB containing 0.08% BSA) and diluted for use.

2.4. Preparation of capture pad

Nitrocellulose membrane UniSart^{TM} CN 140 was cut into strips (25 mm width). The procedures of immobilization of antibodies were shown: (1) the membrane was coated with anti-PAP monoclonal antibody (M01010921) in a volume of 1 μl containing 200 ng antibody as test lines per 3 mm nitrocellulose (NC) membrane (the width of the test-strip) at position 35 mm, 38 mm, 41 mm and 44 mm respectively and with goat-anti-mouse monoclonal antibody in a volume of 1 μl containing 500 ng antibody as control line at position 47 mm on the same membrane by BioDot XYZ 3050 Dispensing Platform (Irvine, CA, USA), (2) the coated test strips were dried at 37 °C for 15 min in constant temperature oven, (3) the remaining protein-binding sites of the membrane were blocked by immersing the strips in PB containing 1% BSA at 37 °C for 30 min, and (4) the test strips were washed with PB and dried at 37 °C for 15 min. The coated strips were stored in a desiccator at 4 °C.

2.5. Manufacture of barcode semiquantitative colloidal gold immunochromatographic strip

Barcode-styled PAP lateral-flow immunostrip used in this study was shown in Fig. 1, which is composed of four zones. The first was sample loading zone covered with a polyester membrane (Grade 7589) in order to promote the even distribution of sample volume over NC membrane. The second zone was conjugate pad, a polyester membrane (Grade 7589) impregnated with labeled colloidal gold by anti-PAP monoclonal antibody (M01010922). The third zone was the test zone with a section of NC membrane mounted onto a polyvinyl chloride plastic backing support strip (J-B8) with adhesive. In advance, the NC membrane was coated with capture reagents. The fourth zone was an absorbent pad adhered at the end of the test strip to provide a pulling force for driving the migration of sample. The membrane was cut into 0.3-cm-width test-strips by using BioDot CM4000 guillotine cutting system (Irvine, CA, USA) for use in the assay.

3. Results and discussion

3.1. Optimal condition for conjugation between colloidal gold and antibody

Gold colloids were prepared by controlled reduction of gold chloride with sodium citrate. The size of the colloidal gold particles

was directly dependent on the amount of trisodium citrate used in its preparation process. In order to acquire gold colloids with higher concentration, the initial concentration of hydrogen tetrachloroaurate (III) tetrahydrate had been doubled. The strength of color showing was closely related to the size and quality of the colloidal gold particles (Fig. 2). It was found that if the diameter of gold particles were small (<10 nm) it was hard to indicate a clear and bright color. It was also found that gold particles with large diameter (>40 nm) were unstable, self-coagulation occurred. For this study, the optimize size selection of diameter for colloidal gold particle was 20 nm. The pH of colloidal gold solution for labeling antibody was adjusted pH 8.2 with 0.1 M K_2CO_3 for monoclonal antibody. For conjugation, antibody was directly absorbed on colloidal gold particle surface, mediated mainly by London-van der Waals force and hydrophobic interaction [16].

Optimal conditions of antibody concentration for the coating could be determined by comparing the adsorption at 580 nm. When increasing the amount of antibodies, D_{580} increases, reaches a maximum, and starts to decline, reaching the plateau that corresponds to saturation of the particles by immobilized antibodies (Fig. 3). The optimal antibody to stabilize 1 ml of gold colloidal suspension was approximately 5 μg anti-PAP antibody.

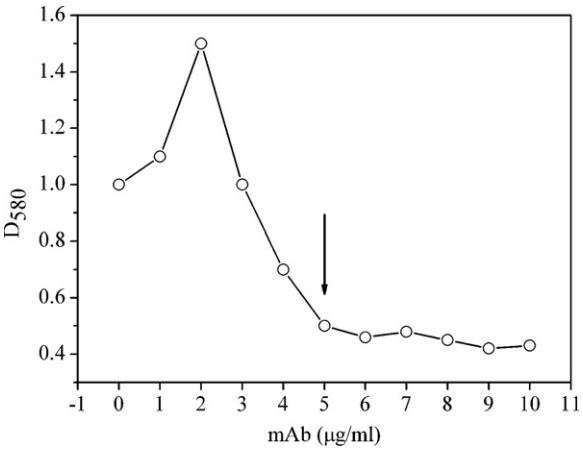

Fig. 3. Concentration dependence of colloidal gold optical density at 580 nm after adding different concentrations of anti-PAP antibody for the choice of the optimal concentration for conjugation.

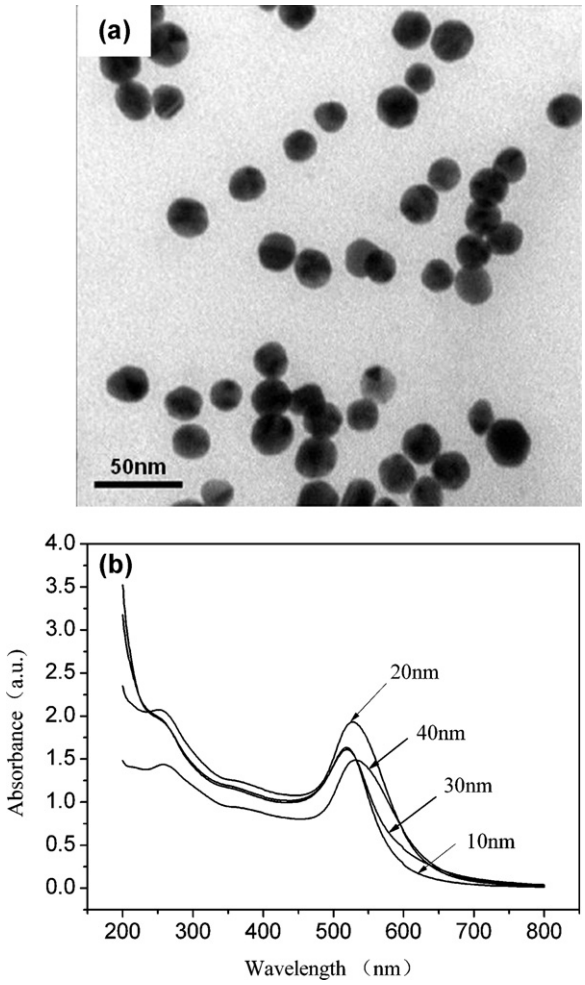

Fig. 2. (a) TEM image of 20 nm diameter gold nanoparticles. Images were obtained using Hitachi H-7100 electron microscope under accelerating voltage 100 kV and enlargement 200,000 and (b) UV–vis spectrum of gold nanoparticles with different diameters.

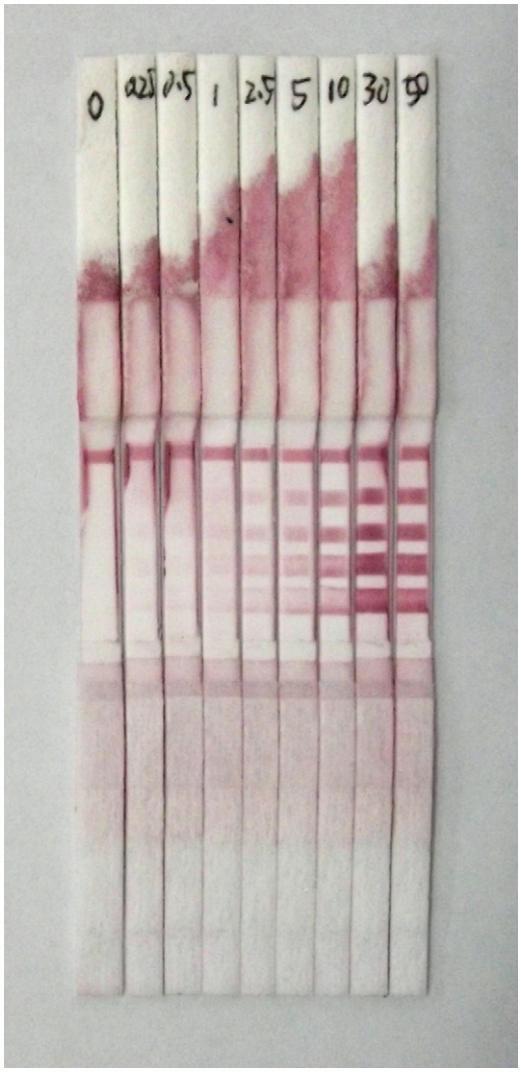

Fig. 4. Detection of PAP in the spiked serum by barcode PAP immunostrip. PAP concentrations in the samples (from left to right): 0, 0.25, 0.5, 1.0, 2.5, 5.0, 10, 30 and 50 ng/ml.

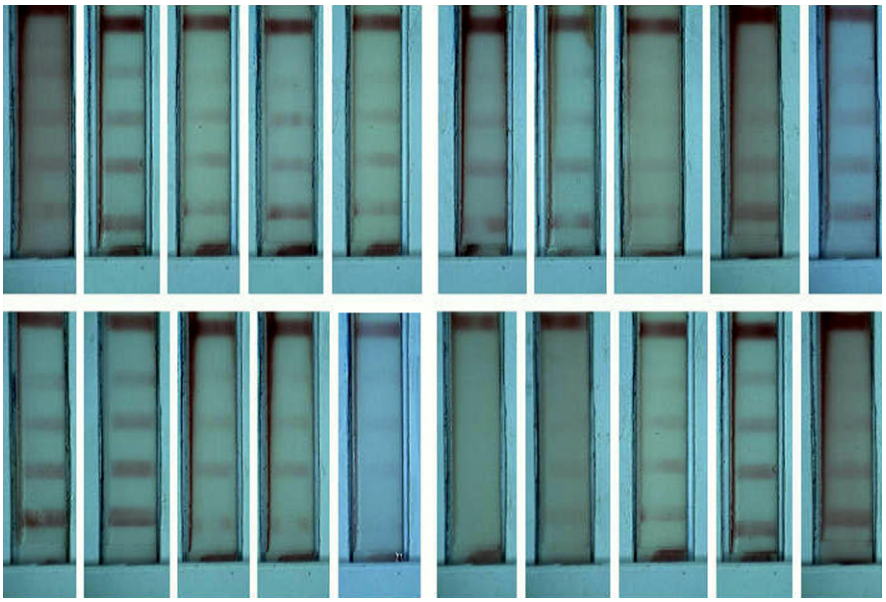

Fig. 5. Use of the barcode-styled PAP immunostrips to assay clinical serum samples. Twenty from sixty-five results were shown.

3.2. Performance of lateral-flow assay

The performance of the barcode semiquantitative colloidal gold immunochromatographic strip relied on sandwiched assay formats, where PAP species were sandwiched between the primary anti-PAP antibody–colloidal gold conjugate and the secondary anti-PAP antibody immobilized on the NC membrane (Fig. 1). The lateral flow immunoassay procedures were shown in the following. Some sample solution were added to the sample pad and allowed to flow through the conjugate pad. By these means the conjugate dissolved and was pulled to flow along the NC membrane together with the sample solution and migrated by capillary toward the test lines (T1, T2, T3, T4) and control line (C). If PAP was present in the sample, it bound on to the antibody capture lines on the strip together with the antibody–colloidal gold conjugate. As a result, several pink color lines appeared on the capture zones of the strip if the PAP content was above the detection limit of the device. Any excess unbound conjugate produced a pink color on the control line, this being an indication of the technical validity of the test. Finally, the overall solution reached the absorbent pad in less than 10 min. Then the strip was immediately washed by dropping a solution (PB containing 0.15 M NaCl and 0.05% Tween 20) on the sample pad. After a 10 min incubation period, the color intensity of the antibody capture lines was firstly assessed visually with the naked eye and determined by densitometry analysis after the strips had fully air-dried. In order to do this, grey scale digital scanned images of the strips were recorded using a luminescent image analyzer LAS-1000 plus (Fujifilm, Tokyo, Japan) and the intensity of the capture lines integrated using Image Reader LAS-1000 Lite for LAS-1000 plus version 1.3. The total assay time was about 30 min.

PAP-free serum was spiked with 0–50 ng/ml purified human PAP to construct a calibration curve. Different sample volumes from 0 to 100 μl have been tested. Eighty microliters sample was chosen as the optimal sample volume to solubilize the detector reagent and 100 μl PBT solution (PB containing 0.15 M NaCl and 0.05% Tween 20) to wash away unbound detector reagent from the NC membrane. The results were obtained by visual examination (Fig. 4). The semi-quantitative value of the specimen was determined by visual comparison of specimens with the standard curve. In all cases the color intensity was directly proportional to the PAP concentration in the sample. At the same time, a standard curve from 0 ng/ml to 30 ng/ml was produced, and the linear regression equation between LAS-1000 Signal (A.U.) and PAP concentrations (ng/ml) is $y = 0.9398(\pm 0.0073)x - 0.00356(\pm 0.02449)$ ($r = 0.9998$). The limit for analysis obtained from the standard curve was 0.25 ng/ml. It was the PAP concentration corresponding to a signal 3 SD above the mean ($n = 20$) for a sample in which PAP was absent.

3.3. Assay precision

The precision study was performed according to the EP5-T2 protocol [17]. Two different human serum panels with a normal and a pathologic concentration were analyzed to assess intraassay precision by running 20 replicates of each sample panel in a single analytical run. Interassay precision was evaluated by measurement of the two serum panels in replicates of two at two separate times for consecutive 10 days, using different lot of PAP immunostrip per day. Imprecisions were evaluated by calculating corresponding CVs using SPSS Statistics V19.0.1 (IBM Corporation). The intraassay CV was 8–10%, and the interassay CV was 10–15% (Table 1).

3.4. Method comparison

The reliability of the test strip immunoassay was determined by carrying out the test with the human samples and analyzed by strip test and instrumental analysis. Sixty-five human samples with PAP concentrations between 0.25 and 40 ng/ml, as assessed with the conventional ELISA (Diagnostic Automation, Inc., Calabasas, CA), were re-evaluated using the newly developed lateral-flow assay as shown partially in Fig. 5. A good correlation between the conven-

Table 1
Imprecision of the PAP immunochromatographic assay.

	Sample	n	PAP (ng/ml)	CV (%)
Intraassay	serum	20	1.35 ± 0.22	9.43
	serum	20	25.6 ± 1.35	8.57
Interassay	serum	20	1.42 ± 0.32	12.5
	serum	20	24.8 ± 1.44	10.3

Note: Intraassay precision was assessed in a single analytical run by analysis of 20 replicates of two human serum samples. The PAP concentrations of the two samples were measured 20 times over a period of 10 consecutive days to calculate interassay variation. Data are means ± SD.

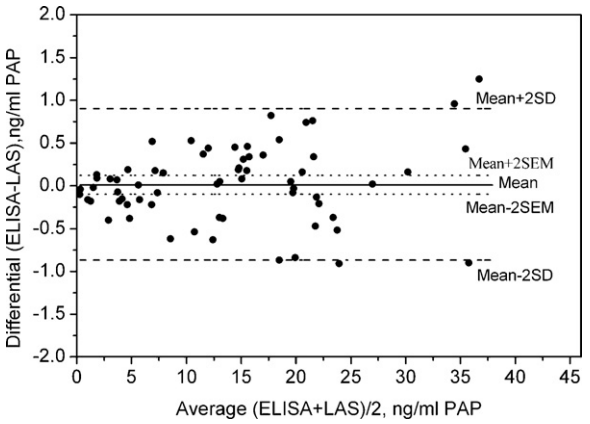

Fig. 6. Bland–Altman plots of a method comparison for serum between the immunochromatographic assay and the routine ELISA. Mean difference (solid line), 95% confidence interval for the mean difference (mean ± 2SEM, doted line), and limits of agreement (mean ± 2SD, dashed line) are shown.

tional ELISA and the newly developed lateral-flow assay was found ($y = 0.9894x + 0.0203$, $r = 0.9687$). Moreover, Agreement between the two methods was analyzed in a different plot according to Bland and Altman [18,19]. According to Bland and Altman plot (Fig. 6), the 95% confidence interval (Mean ± 2SEM, i.e. −0.09 and 0.13 ng/ml) included zero, therefore there was no evidence of systematic bias. With respect to the precision, the scatter of the differences was found to increase as the average increased, but the limits of agreement (Mean ± 2SD, i.e. −0.86 and 0.91 ng/ml) are acceptably small. We conclude that there is a good agreement between the methods.

3.5. Stability test

To assess the shelf life stability of unopened test strips, test strips were stored at 50 °C as well as stored at 5 °C as a control. After 1, 2, 3, 4 and 5 weeks of storage, test strips were tested with blood adjusted to plasma PAP levels of 1.0, 5.0, 12, and 30 ng/ml. Sixteen replicate PAP values were obtained with each storage condition, test strip lot, and blood aliquot. The data at each checkpoint were evaluated by comparing the mean PAP value obtained using test strips stored at 50 °C to the mean PAP value obtained using test strips stored at 5 °C. The stability change undergone by the 50 °C samples over time, relative to the 5 °C samples, is plotted in Fig. 7. Arrhenius modeling was used to determine how the time scales for the 50 °C accelerated stress conditions related to the time scale of the 30 °C condition. This analysis indicated that to predict the

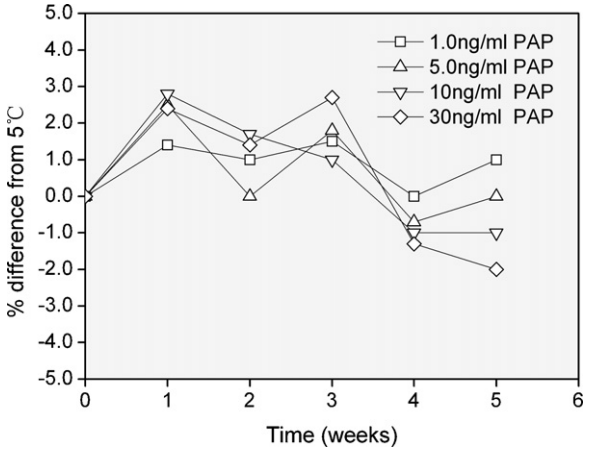

Fig. 7. The relationship between percent changes in PAP results and time of storage at 50 °C at each of four PAP concentrations.

stability at 30 °C, the storage time at 50 °C should be multiplied by 11. Thus 5 weeks at 50 °C is equivalent to approximately 55 weeks (about one year) at 30 °C. Thus the test-strips stored air-dried at room temperature were stable up to one year without significant loss of activity.

4. Conclusions

In this study, we present the successful development of the rapid and barcode semiquantitative lateral-flow assay for PAP in serum or plasma samples. The quantitative test uses a small quantity of serum sample and returns results within 30 min. Results obtained with the test in the clinics underline the good performance presented in this paper. Compared with centralized laboratory testing, it provides for rapid clinical decision-making by reducing the time spent on transporting samples and retrieving data. To further reduce assay time and simplify the assay procedure, a rapid immunochromatographic strip for qualitative determination of PAP in whole blood samples is now under development. It is derived from the serum lateral-flow test but used a plasma separation membrane instead of a sample pad.

Acknowledgements

This work was supported by the National High Technology Research and Development Program of China (Research grant no. 2007AA022006) and the Scientific and Technical Development Project of Guangxi (No. Guikeneng 0992028-8). The authors wish to thank the nurses and staff of Urology Department in the Second Xiangya Hospital of Central South University for collecting blood samples.

References

[1] L.A.G. Ries, D. Melbert, M. Krapcho, D.G. Stinchcomb, N. Howlader, M.J. Horner, A. Mariotto, B.A. Miller, E.J. Feuer, S.F. Altekruse, D.R. Lewis, L. Clegg, M.P. Eisner, M. Reichman, B.K. Edwards, SEER Cancer Statistics Review, 1975–2005, National Cancer Institute, Bethesda, MD, 2008.
[2] I.M. Thompson, D.K. Pauler, P.J. Goodman, C.M. Tangen, M.S. Lucia, H.L. Parnes, L.M. Minasian, L.G. Ford, S.M. Lippman, E.D. Crawford, J.J. Crowley, C.A.J. Coltman, Prevalence of prostate cancer among men with a prostate-specific antigen level ≤4.0 ng/ml, N. Engl. J. Med. 350 (2004) 2239–2246.
[3] G.L. Andriole, E.D. Crawford, R.L.r. Grubb, S.S. Buys, D. Chia, T.R. Church, M.N. Fouad, E.P. Gelmann, P.A. Kvale, D.J. Reding, J.L. Weissfeld, L.A. Yokochi, B. O'Brien, J.D. Clapp, J.M. Rathmell, T.L. Riley, R.B. Hayes, B.S. Kramer, G. Izmirlian, A.B. Miller, P.F. Pinsky, P.C. Prorok, J.K. Gohagan, C.D. Berg, P.P. Team, Mortality results from a randomized prostate-cancer screening trial, N. Engl. J. Med. 360 (2009) 1310–1319.
[4] A. Taira, G. Merrick, K. Wallner, M. Dattoli, Reviving the acid phosphatase test for prostate cancer, Oncology (Williston Park) 21 (2007) 1003–1010.
[5] S. Bührer-Sekula, F.F.V. Hamerlinck, T.A. Out, L.G. Bordewijk, P.R. Klatser, Simple dipstick assay for semi-quantitative detection of neopterin in sera, J. Immunol. Methods 238 (2000) 55–58.
[6] O.P. Crabbe, M. Salden, J. Wichers, C. Van Peteghem, F. Kohen, G. Pieraccini, G. Moneti, Preliminary evaluation of a lateral flow immunoassay device for screening urine samples for the presence of sulphamethazine, J. Immunol. Methods 278 (2003) 117–126.
[7] S.V. Bannur, S.V. Kulgod, S.S. Metkar, S.K. Mahajan, J.K. Sainis, Protein determination by Ponceau S using digital color image analysis of protein spots on nitrocellulose membranes, Anal. Biochem. 267 (1999) 382–389.
[8] J.H. Cho, S.M. Han, E.H. Paek, I.H. Cho, S.H. Paek, Plastic ELISA-on-a-chip based on sequential cross-flow chromatography, Anal. Chem. 78 (2006) 793–800.
[9] F.J. Hayes, H.B. Halsall, W.R. Heineman, Simultaneous immunoassay using electrochemical detection of metal ion labels, Anal. Chem. 66 (1994) 1860–1865.
[10] J.H. Kim, J.H. Cho, G.S. Cha, C.W. Lee, H.B. Kim, S.H. Peak, Conductimetric membrane strip immunosensor with polyaniline-bound gold colloids as signal generator, Biosens. Bioelectron. 14 (2000) 907–915.
[11] M.F. Cardosi, S.W. Birch, C.J. Stanley, A. Johannsson, A.P.F. Turner, An electrochemical immunoassay for prostatic acid phosphatase incorporating enzyme amplification, Am. Biotechnol. Lab. 7 (1989) 50–58.
[12] S.C. Lou, C. Patel, S. Ching, J. Gordon, One-step competitive immunochromatographic assay for semiquantitative determination of lipoprotein(a) in plasma, Clin. Chem. 39 (1993) 619–624.

[13] J.H. Cho, S.H. Paek, Semiquantitative, bar code version of immunochromatographic assay system for human serum albumin as model analyte, Biotechnol. Bioeng. 75 (2001) 725–732.
[14] W. Leung, C.P. Chan, T.H. Rainer, M. Ip, G.W.H. Cautherley, R. Renneberg, InfectCheck CRP barcode-style lateral flow assay for semi-quantitative detection of C-reactive protein in distinguishing between bacterial and viral infections, J. Immunol. Methods 336 (2008) 30–36.
[15] G. Frens, Controlled nucleation for regulation of particle-size in monodisperse gold suspensions, Nat. Phys. Sci. 241 (1973) 20–22.
[16] G.T. Hermanson, A.K. Mallia, P.K. Smith, Immobilized Affinity Ligand Techniques, Academic Press, San Diego, CA, 1992.
[17] NCCLS, Evaluation of precision performance of clinical chemistry devices; Tentative Guideline. NCCLS Document EP5-T2, 2nd ed., National Committee for Clinical Laboratory Standards, Villanova, PA, (1992).
[18] J.M. Bland, D.G. Altman, Statistical method for assessing agreement between two methods of clinical measurement, Lancet 327 (1986) 307–310.
[19] J.M. Bland, D.G. Altman, Measuring agreement in method comparison studies, Stat. Methods Med. Res. 8 (1999) 135–160.