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Colloidal Nanogold-Based Immunochromatographic Strip Test for the Detection of Digoxin Toxicity

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Abstract Digoxin is widely used as a cardiac glycoside drug in the treatment of various heart conditions. Because it is a toxic drug, it should be regularly monitored in the serum of patients under treatment. In this study, colloidal nanogold is synthesized and the preparation of nanogold-labeled monoclonal antibody probe to digoxin is described under optimal conditions. In addition, an immunochromatographic (IC) method for digoxin analysis employing nanogold-labeled probe is developed. With this technique, it requires only 5 min to complete the quantitative detection of digoxin. The detection time is decreased 20–30 times in comparison to radioimmunoassay (RIA). The sensitivity to digoxin was about 2 ng/ml by naked eye, which is within the therapeutic and toxic ranges of digoxin. The results of serum samples obtained by IC strip were in agreement with those obtained by RIA. The IC strip was sufficiently sensitive and accurate to be used for the rapid detection of digoxin in serum samples.

Keywords Digoxin · Immunochromatography · Colloidal nanogold · Monoclonal antibody

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Introduction

Digoxin has been used as a cardiac glycoside in clinical treatments for more than 200 years [1]. It is currently utilized as an antiarrhythmic drug and is generally prescribed to increase the adequacy of circulation in patients with congestive heart failure. Digoxin is also employed to decrease the ventricular rate when atrial fibrillation and flutter exists. The prime mechanism of digoxin action in heart failure is the inhibition of sodium–potassium adenosine triphosphatase (Na–K-ATPase) enzyme. The suppression of this enzyme in myocardial cells causes an increase in heart-muscle contractility. However, it is recently known that the benefit of digoxin in heart failure also results partly from the inhibition of this enzyme in non-cardiac tissues. In other words, digoxin reduces sympathetic activities in vagal afferent nerve fibers and reduces renin–angiotensin–aldosterone system activities in renal tubules [2].

It is important to measure digoxin concentration in the serum of digitalized patients. The importance of this issue is related to its narrow therapeutic range (0.8–1.9 ng/ml) of digoxin, i.e., toxicity may be encountered in a concentration as low as 2.0 ng/ml [3]. Moreover, symptoms of digoxin toxicity are similar to symptoms of patients with congestive heart failure who need the prescription of this drug. The symptoms could also be dissimilar in different body systems; for example, the serum digoxin level may be efficiently changed by drugs or clinical status such as renal failure. Heart failure patients usually have a digoxin toxicity problem which is the result of diuretic therapy and secondary hyperaldosteronism [4]. The level of digoxin in serum can be measured by means of several methods including chromatography and immunochemical assays.

High performance liquid chromatography (HPLC), the confirmatory chromatography method, is used for the measurement of some analytes including digoxin [5]. Although HPLC has the advantages of high sensitivity and specificity, it is mostly laborious, expensive, and must be done by experienced technicians. HPLC, however, requires an extraction step sample preparation and an enrichment step prior to its determination. Therefore, they are not suitable for emergency cases.

Immunoassays are demonstrated to be simple, rapid, and cost effective and can be used for the analysis of digoxin in numerous biological samples [3, 6, 7]. Enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) are the most common immunoassay methods which have been used for the determination of digoxin toxicity in serum. Although these methods provide the advantages of sensitivity and specificity and require a small sample volume for analysis, they often require long reaction times and involve multiple steps. The utilization of these immunoassays has been confined to laboratories equipped with tools and devices for performing analyses.

In emergency cases, an ideal alternative could be on-site test strip, a kind of immunochromatographic technique, which does not depend on instrumentation and is also rapid, sensitive, specific, cheap, and easy to interpret [8, 9]. On-site test strip has been extensively used for many qualitative and semi-quantitative assays such as Aflatoxin B1 [10], hepatic disease [11], hormones, toxins, viruses, bacteria and parasitic antigens [9, 12–14] as well as drug abuse and protein markers [11, 15] and so on.

The possibility of direct antigen–antibody detection by naked eye arises from the fact that test strip labeling substances, such as highly colored colloidal gold particles and latex beads [9], create a change in color density according to the analyte [16]. The ability to execute such diagnostic tests at a location far from the laboratory would be highly attractive for its speed and economy cost. The competitive IC strip can be used for detection of low molecular weight antigen. In this type of assay, a detector reagent is often the antibody coupled to gold particle as a tracer molecule. The capture zone is usually analytes conjugated to a carrier

protein or antibody immobilized on the nitrocellulose membrane. In one-step competitive IC test strip, as the test sample flows up through the absorbent device, the free analyte in the specimen competes with immobilized antigen conjugate in the test zone by binding to the monoclonal antibody that has been conjugated with the colloidal gold nanoparticles [17–19].

In 1990, Choi et al. introduced a colloidal gold immunochromatography assay (ICA) for the quantitative measurement of digoxin in serum. The visual detection limit for the test strip was 1 μg/ml and it required 2.0 min to provide the results. Therefore, a more sensitive and rapid assay may be necessary in emergency conditions for detecting the level of digoxin toxicity.

In the present work, we describe the development of a novel sensitive, rapid, and accurate IC test strip that could be applied for the detection of digoxin by an inexpert person and the result could be obtained rapidly.

Materials and Methods

Materials

Chloroauric acid ($HAuCl_4$), mouse monoclonal anti-digoxin antibody (clone DI-22), tween-20, bovine serum albumin (BSA), anti-mouse IgG–horse radish peroxidase (IgG–HRP), protein G column, ethylene glycol, digoxin, sodium azide, sodium periodate, sodium borohydride, and sodium citrate were purchased from Sigma Chemical Company (St. Louis, MO, USA). ELISA plates (96 wells) and other plastic wares were purchased from Nunc (MAX-ISORP, Roskilde, Denmark). High-flow nitrocellulose membrane, glass fiber pads as well as sample and absorption pads were obtained from Schleicher and Schuell Millipore Co. (Dassel, Germany). A Camag Linomat 5 automatic TLC sampler (Switzerland) was used. Digoxin–bovine serum albumin (Dig–BSA) was produced in our laboratory.

Methods

Preparation of Digoxin–Protein Conjugates Twenty one milligrams of digoxin (0.027 mmol) was suspended in 0.67 ml of 95% ethanol and was stirred for 30 min. $NaIO_4$ was added (0.67 ml of 0.1 M) dropwise during 60 min to the digoxin suspension. The mixture was stirred for 30 min and then 0.02 ml of ethylene glycol 1 M was added to the oxidized digoxin solution. After continuously mixing it up for 5 min, 0.67 ml of BSA solution (18.6 mg/ml in 0.1 M sodium bicarbonate, pH 8.2) was added into the mixture dropwise. Adjusting the pH in the range 9.3–9.5 with 1 M Na_2CO_3 (two drops), stirring was carried on for 1 h at room temperature. Six-tenths milliliter of 0.4 M $NaBH_4$ solution was added dropwise and was stirred for 30 min at 4 °C. The reaction mixture was dialyzed against 0.1 M carbonate buffer, pH 9.3 [20].

Synthesis of Colloidal Gold The aqueous solution of chloroauric acid (0.01% *w*/*v* $HAuCl_4$) and sodium citrate solution (1% *w*/*v*) were prepared. Chloroauric acid solution (100 ml) was heated to boiling with electric heating in an Erlenmeyer flask with a magnetic stirrer, and then 3 ml of the aqueous 1% sodium citrate solution was added to the flask while stirring rapidly; the mixing speed was 1,000 rpm. The reaction time was 9–10 min; during this time, the color of the solution was changed to wine red.

Preparation of Colloidal Gold–mAb Probe Colloidal gold (18–20 nm in diameter), prepared in the previous stage, was used for the conjugation of IgG. The colloidal gold

solution (1% *w*/*v*) was adjusted to pH 8.5 with 0.1 M Na_2CO_3. Then, 1.5 mg digoxin monoclonal antibody (4.3 mg/ml in phosphate buffer, 10 mM, pH 7.2) was added to the 50 ml pH-adjusted colloidal gold solution drop by drop. The mixture was smoothly mixed for 60 min. Then, we added 2 ml of 10% BSA solution in order to block the residual surface of the nanocolloidal gold particles. The obtained solution was stirred for 15 min and then was centrifuged at 15,000 rpm for 45 min at 4 °C. After centrifugation, the pellet was suspended in 50 ml dilution phosphate buffer (10 mM buffer pH 7.2 containing 1% *w*/*v* BSA). The resultant solution was then centrifuged for the second time under the same condition. The pellet was resuspended in 5 ml dilution phosphate buffer (10 mM sodium phosphate pH 7.2 containing 1% *w*/*v* BSA and 0.1% sodium azide) and the optical density was adjusted to 8.0 at 520 nm with the dilution buffer. This anti-digoxin IgG coated colloidal gold probe was stored at 4 °C.

Characterization of Conjugates The formation of colloidal gold was screened using UV–vis spectroscopy method (200–700 nm) by means of a double-beam spectrophotometer (Shimadzu, model 1.70, GBC) running at 1 nm. Antibody–colloidal gold conjugates were also monitored just after the addition of the antibody. Finally, 1 ml of the conjugated solution was screened after adding 0.1 ml anti-mouse IgG (12 mg/ml) and the agglutination.

The relative stability and activity of the conjugate probe was determined using an incompetitive indirect ELISA. For this purpose, microtiter plates were coated with 100 μl of Dig–BSA (5 μg/well) in phosphate-buffered saline (PBS, 10 mM, pH 7.2) overnight at 37 °C. The following day, the plates were blocked with PBS containing 3% skim milk (blocking buffer) for 1 h at 37 °C. Following the blockade of the non-specific binding sites, the wells were treated with the colloidal gold anti-digoxin mAb conjugate and free antibody at a concentration of 1 μg/well. This was incubated at 37 °C [bovine serum albumin was used as non-specific binding (NSB)]. The residual binding was detected with anti-mouse HRP conjugated antibody and expressed as a percentage of the absorbance value given by a control.

Preparation of IC Test Strip Lateral flow test strip was built according to the method of Paek et al. [9]. The sample pad was saturated with a buffer (pH 7.4) containing 20 mM sodium phosphate, 1% (*w*/*v*) BSA, 0.5% (*v*/*v*) tween-20, and 0.05% (*w*/*v*) sodium azide and was dried for 1 h at 50 °C. An absorption pad was used without treatment. The conjugate pad (glass fiber membrane) was treated with a 5% (*w*/*v*) sucrose solution in water and was then dried. Then, 0.7 μl/strip gold-labeled antibody probe (without dilution) was applied to the sucrose-treated glass fiber membrane to be used as a conjugate pad. The conjugate pad was dried for 30 min at 37 °C. Subsequently, 1 μl goat anti-mouse antibody (1 mg/ml) and a Dig–BSA antigen (2 mg/ml in phosphate-buffered saline, 10 mM, pH 7.2) were coated onto a nitrocellulose membrane as two distinct sectors with Camag Linomat 5 automatic TLC sampler, one for controlling and the other for testing; it was dried for 30 min at room temperature. The nitrocellulose membrane binds the antibody and the antigen by an electrostatic mechanism. The highly strong dipole of the nitrate ester interacts with the strong dipole of the peptide bonds of the antibody and the antigen. The purpose of drying the membrane at room temperature is to fix the antibody and the antigen to the nitrocellulose.

The blotted nitrocellulose membrane, the absorption pad, the conjugated pad, and the sample pad were assembled as the test strip. Then, the strips with 70 mm in length and 5 mm width were cut. The strips were sealed in a plastic bag and stored under dry conditions at 4 °C until use. One hundred microliters of digoxin sample was applied onto the sample pad to evaluate the result.

Furthermore, in the other part of this study, the nitrocellulose membranes with the immobilized Dig–BSA antigen and goat anti-mouse antibody were soaked in PBS

containing various concentrations of sucrose and dried at 37 °C. The stability of the immunostrips treated with various concentrations of sucrose (3%, 5%, and 8%) was determined for 1 month after storage at 60 °C and at room temperature for 1 year and was compared with that prepared in the absence of sucrose.

Sample Collection and Analysis

Human blood samples were obtained from the laboratory of Endocrinology and Metabolism Research Center (EMRC), Tehran University of Medical Sciences. The study protocol was approved by ethics committee of EMRC.

Human blood samples were transferred into a test tube. After incubation at room temperature for 1 h, the test tube was centrifuged for 10 min at 1,200 ×*g*. The supernatant was loaded into a vial and stored frozen at −20 °C until use.

The precise concentration of digoxin in the 40 different serum samples were measured by an automated radio immunoassay system (RIA), and their results were compared with the IC test strip.

Results

Calculation of the Digoxin Moieties Bound to BSA

The number of digoxin molecules bound to each BSA is calculated according to the following equation: 1 mg per 5 ml Dig–BSA conjugate, Dig and BSA was prepared. Then, the light absorption of the solutions at 388 was measured:

$$A_{388}(\text{Dig}-\text{BSA})=0.32 \qquad A_{388}(\text{Dig})=1.3 \qquad A_{388}(\text{BSA})=0.09.$$

The difference between the light absorption of digoxin bound to BSA and BSA is measured using the following formula:

$$A_{388}(\text{Dig}-\text{BSA})-A_{388}(\text{BSA})=0.23.$$

The molecular weight of digoxin and BSA is 780 g and 66,000 Da, respectively.

Therefore, we can calculate extinction coefficient and molar concentration of digoxin bond to BSA as follows:

$$\begin{aligned} A &= \varepsilon C 1 \\ 1.3 &= \varepsilon \times \tfrac{1}{780\times 5} \times 1 \Rightarrow \varepsilon = 5,120 \\ C &= \tfrac{A}{\varepsilon l} \Rightarrow C = \tfrac{0.23}{5,120\times 1} = 4.49\times 10^{-5} \end{aligned}$$

The molar concentration of BSA is:

$$C=\frac{1}{66,000\times 5}=3.03\times 10^{-6}$$

Finally, the number of digoxin molecules bound to each BSA was calculated as follows:

$$\text{Number of digoxin molecules bound to each BSA}=\frac{4.44\times 10^{-5}}{3.03\times 10^{-6}}=14.81$$

This means that 14 or 15 digoxin molecules are conjugated to each BSA.

Characterization of Colloidal Gold Particles

Chloroauric acid was reduced to gold atoms by sodium citrate and many of gold atoms were nucleated into nanogold particles. Figure 1 illustrates the size of gold particles obtained by transmission electron microscopy measurement. The achieved average diameter for the colloidal gold was 18–20 nm.

Optimal Condition Studies for Conjugation

For providing a stable conjugate of colloidal gold and mAb, a minimum amount of the antibody must be used. Therefore, a preliminary serial dilution experiments were performed to quantitatively determine the minimal antibody concentration sufficient for having a strong absorption between gold and antibody conjugates.

This minimum amount was determined by adding 0.5 ml of 10% NaCl to 1 ml colloidal gold particles containing different amounts of mAb (0.005–0.05 mg/ml) and was shaken for 10 min. After 5 min, the absorption was measured at 520 and 580 nm (A520–A580). The minimum amount of mAb was evaluated by the change of color from reddish to blue. The optimum concentration of mAb for colloidal gold labeling was the lowest concentration of mAb solution that did not change the color. In our experiment, 30 μg of the antibody was determined as the minimal antibody concentration for the stabilization of colloidal gold. Figure 2 shows the light absorption of the conjugate at different antibody concentrations.

Characterization of Antibody–Gold Conjugates

The UV–vis spectra of the colloidal gold and conjugates, prepared as described previously, is depicted in Fig. 3 where the spectra of the as-prepared colloidal gold solution, antibody–colloidal gold conjugate, and the conjugate solution after agglutinating with goat anti-mouse antibody IgG were compared. The peak at ~519 nm in curves is caused by the surface plasmon resonance of colloidal gold particles [10]. After the addition of the antibody, due to the interaction of the antibody with colloidal gold particles, the surface plasmon band was

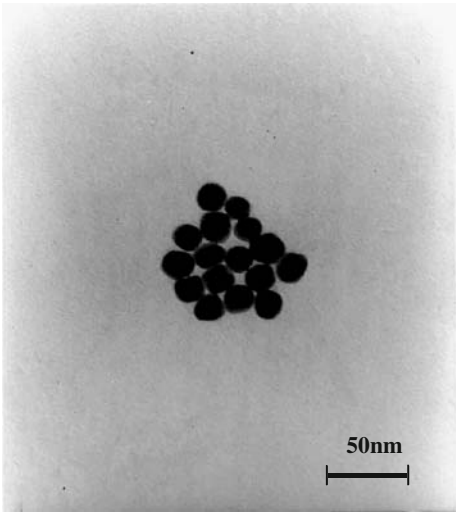

Fig. 1 Colloidal gold observed by transmission electron microscope. The diameter of particles was 18–20 nm

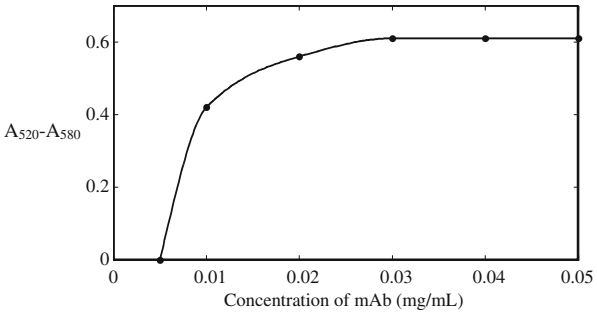

Fig. 2 Light absorption of conjugation at different antibody concentrations (0.005–0.05 mg/ml)

expanded and red shifted. The agglutination of the gold probe with goat anti-mouse antibody IgG reduces the surface of nanoparticles; consequently, the surface plasmon resonance is decreased.

The reactivity of the antibody after absorption onto the nanoparticle surface is the essential aspect of the protein–gold probe system. The stability of the conjugates and the free antibody incubated at 4 and 37 °C was determined by incompetitive indirect ELISA every 7 days. The immunoreactivity of the free antibody and the probe is indicated by the OD values of negative well minus positive well (5 μg/ml Dig–BSA) at different times. After the incubation of the antibody–colloidal gold conjugate and free antibody at 4 and 37° C for 30 days, the probe was still stable and there was a slow loss in its immunoreactivity. In comparison, the free antibody solution showed a rapid decrease in reactivity and stabilization under the same conditions. Both conjugate probe and free antibody kept 90% and 42% of their residual activity after 30 days under the same conditions. These results indicated that the conjugate probe is more stable than the free antibody (Fig. 4). In addition, the results showed that the immunostrip kept its detective capacity at a concentration of 3% sucrose for at least 1 month after storage at 60 °C and at room temperature for 1 year.

Construction of IC Test Strip

Gold-labeled antibody probe is employed to develop an IC test strip for digoxin. A schematic diagram of the IC test strip is depicted in Fig. 5.

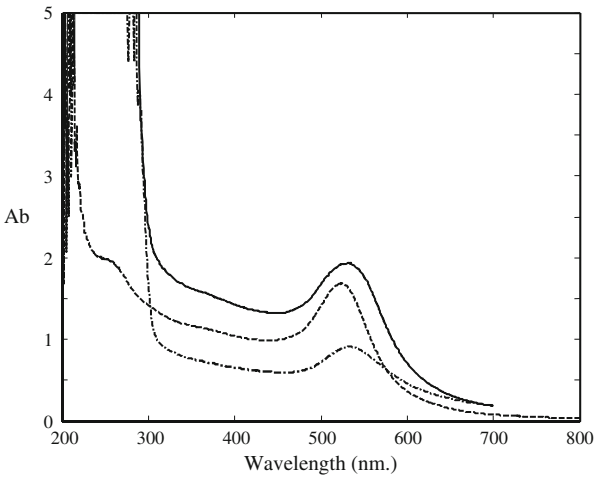

Fig. 3 The UV–vis spectra of the colloidal gold solution before conjugation, antibody–colloidal gold conjugate, and after agglutination with goat anti-mouse antibody. *Dashed* colloidal gold, *solid* Ab–gold conjugate, *dashed-dotted* agglutinated sample

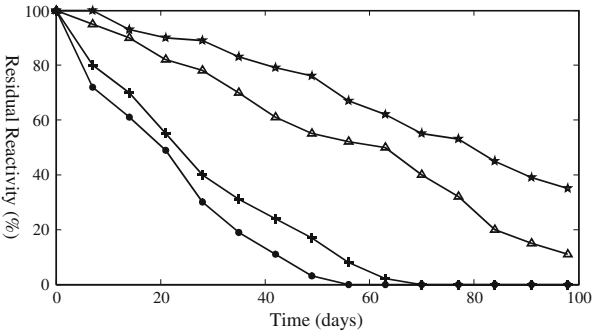

Fig. 4 Stability measurements of Ab–colloidal gold conjugate and free Ab every 7 days by ELISA. ★ Residual reactivity of conjugate probe at 4 °C. Δ Residual reactivity of free Ab at 4 °C. ✚ Residual reactivity of conjugate probe at 37 °C. ● Residual reactivity of free Ab at 37 °C

For developing a sensitive and rapid digoxin IC test strip, various conditions were examined as follows: First, different concentrations of Dig–BSA (0.5, 1, and 2 μg/μl) and goat anti-mouse antibody (0.5, 1, 1.5, and 2 μg/μl) were immobilized on the nitrocellulose membrane as test and control lines. The proper amount of these antigens for the assay was determined at 2 μg/μl and 1 μg/μl for Dig–BSA and goat anti-mouse antibody, respectively. We then examined different volumes of gold-labeled antibody probe (0.5, 0.7, and 1.5 μl/strip) for preparing the conjugate pad. The results suggested that the optimal amount of colloidal gold–mAb was 0.7 μl/strip.

Digoxin toxicity may be encountered in a concentration of 2.0 ng/ml or more because of narrow therapeutic range (0.8–1.9 ng/ml) of digoxin. Therefore, we have to develop a strip that determines the level of digoxin under or equal to 2 ng/ml. The standard compounds were prepared by the dilution of digoxin stock solution (1 mg/ml) with normal serum samples to achieve the final concentrations of 1, 2, and 3 ng/ml. Some conjugate pads with different volumes of gold-labeled antibody probe (1, 0.7, and 0.5 μl/strip) was prepared and used to develop the IC test strip. The IC test strip was tested with these standard compounds and the results were judged by naked eye. The results suggested that the optimal amount of gold-labeled antibody was 0.7 μl/strip (Fig. 6).

Finally, the sensitivity and specificity of the test strip were evaluated. The sensitivity of the test strip was determined by testing digoxin standard samples. The standard compounds were prepared by the dilution of the digoxin stock solution (1 mg/ml) with normal serum samples to the final concentrations of 0, 1, 1.5, 1.8, and 2 ng/ml. Then, these standard samples were examined and judged by naked eye.

Serum samples containing digoxin at above 2 ng/ml represented a visible color on the control line but did not show a color on the result line. Serum samples containing digoxin less than 2 ng/ml represented a visible color on the test line as well as the control line. These results showed that the detection limit of the test strip for monitoring digoxin in serum was 2 ng/ml (Fig. 7). To evaluate the quality of test strip, a number of digoxin-like compounds and other steroids were prepared in the same as digoxin standard. Then, the specificity of the test strip was examined in comparison to theses compounds. As shown in Table 1, two clear red lines were observed in the test and control lines on various concentrations of these compounds. The results have shown that the IC test strip have a high specificity to digoxin without cross-reaction for other compounds.

Comparison of the Qualitative IC Test Strip with the RIA

To study the consistency of the method, IC test strip was used to determine the level of digoxin in the serum of digitalized patients. We performed the test on 40 serum samples including 15 positive samples as well as 25 negative controls and compared the results of

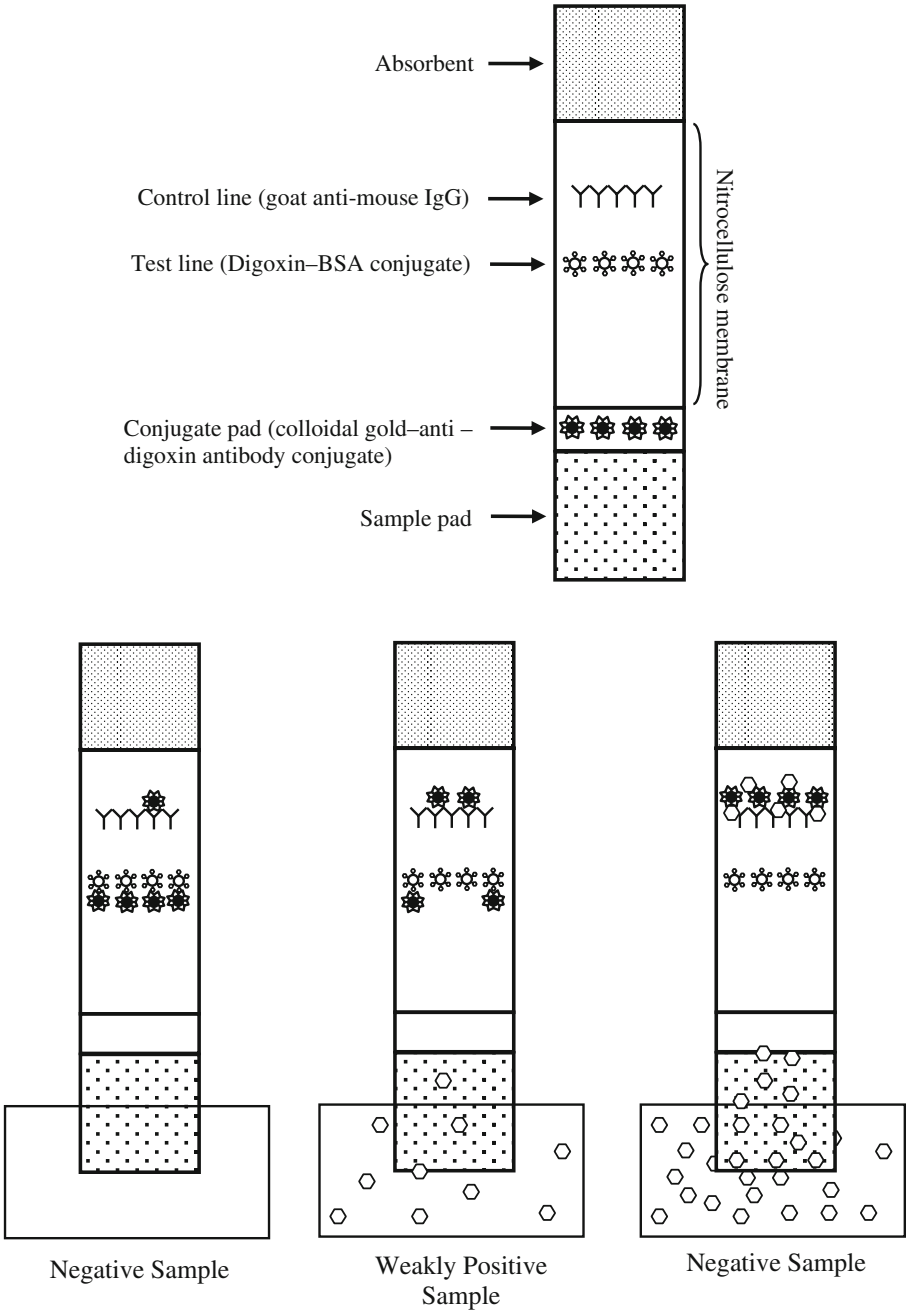

Fig. 5 The schematic description of the IC test strip. The strip consists of a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorption pad. The conjugate pad contains gold-labeled mAb. Nitrocellulose membrane in the detection zone is used as a chromatographic support on which Dig–BSA and goat anti-mouse antibody is immobilized. By visualization, negative (digoxin level equal zero), weakly positive (digoxin level <2 ng/ml), and positive (digoxin level ≥2 ng/ml) samples can be detected by eye

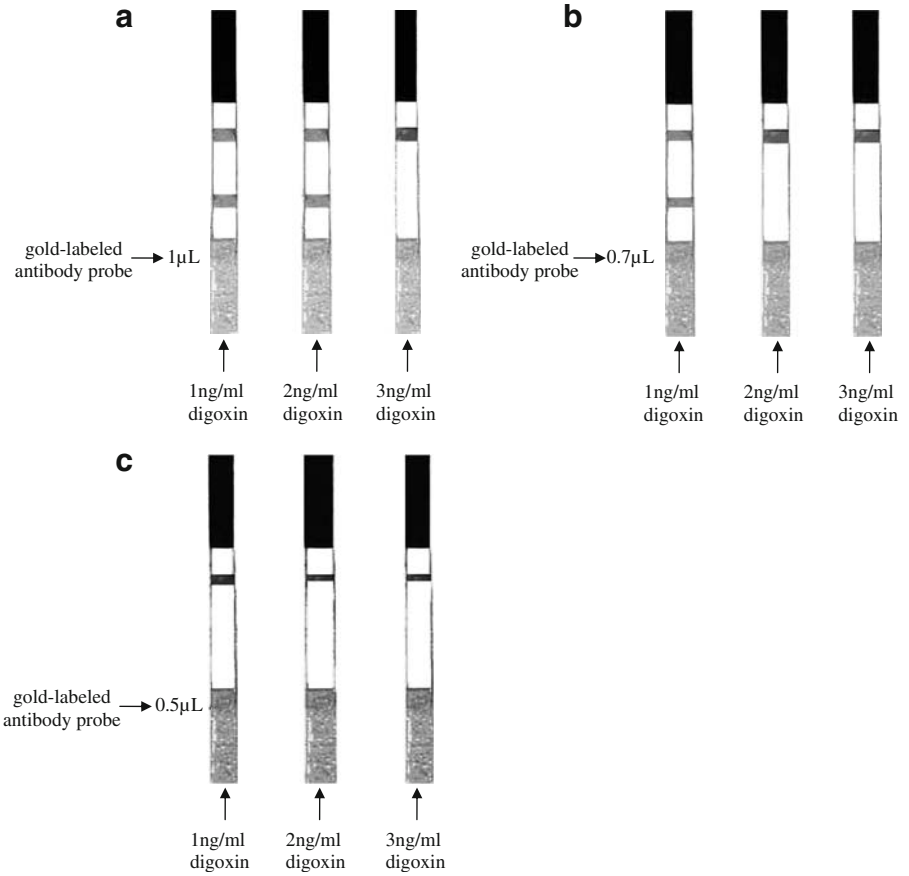

Fig. 6 The effect of different volumes of gold-labeled antibody for developing IC test strip: **a** 1 μl of nanogold-labeled antibody was used; **b** 0.7 μl of nanogold-labeled antibody was used; **c** 0.5 μl nanogold-labeled antibody was used

these tests with those obtained by RIA. In the RIA analysis, 15 serum samples of digitalized patient were found to have digoxin more than or equal to 2 ng/ml and 25 samples were less than 2 ng/ml. The results demonstrated that two methods have an excellent agreement with each other. By visualization only, one negative sample was determined as positive by this test strip. These characteristics make strip assays appropriate applicants for developing a rapid test for digoxin monitoring in emergency conditions.

Discussion

Lateral flow assay or simple form strip assay or ICA has been developed for a while. This technique is based on an IC method that employs antigen–antibody in a new manner to provide a rapid detection of the analyte. Colloidal gold has been generally used as an immunospecific probe for developing IC test strips. The strength of the color is closely related to the size and quality of colloidal gold particles. In this study, nanocolloidal gold with a diameter of 20 nm was chosen to develop IC test strip because colloidal gold

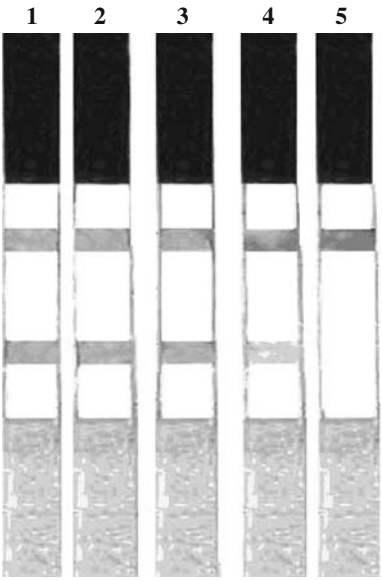

Fig. 7 Test results of working standards with different concentrations of digoxin in serum using the strips. *1* 0 ng/ml, *2* 1 ng/ml, *3* 1.5 ng/ml, *4* 1.8 ng/ml, *5* 2 ng/ml. The test was run three times at room temperature using digoxin standard. Detection limit of IC test strip was determined at 2 ng/ml

particles smaller than 15 nm were found to be too small to produce a strength color. Furthermore, colloidal gold particles larger than 60–70 nm offered a self-aggregation after the particles had been stored at 4 °C for several days [19, 21].

For conjugation, immunoglobulin is directly absorbed on particle surfaces and this process can be accomplished by non-covalent reactions including London–van der Waals force and hydrophobic interactions. The balance between electrostatic repulsion and London–van der Waals attraction among the particles causes the formation of the colloidal gold solution. The strength of the color is closely related to the size and quality of colloidal gold particles. However, on the addition of ionic substances, the attracting force becomes larger than the counteraction and leads to an aggregation and a color change from red (λ_{max} ≈520 nm, A 520) to blue (λ_{max}, A 580) [22]. This instability of colloidal gold particles can be avoided by

Table 1 Cross-reactivity of test strip with related compounds.

Cross-reaction		Concentration (ng/ml)						
		0	1	5	10	50	100	200
Aldospirone	Test line	+	+	+	+	+	+	+
	Control line	+	+	+	+	+	+	+
Cortisol	Test line	+	+	+	+	+	+	+
	Control line	+	+	+	+	+	+	+
Digitoxin	Test line	+	+	+	+	+	+	+
	Control line	+	+	+	+	+	+	+
Estradiol	Test line	+	+	+	+	+	+	+
	Control line	+	+	+	+	+	+	+
Progesterone	Test line	+	+	+	+	+	+	+
	Control line	+	+	+	+	+	+	+
Testosterone	Test line	+	+	+	+	+	+	+
	Control line	+	+	+	+	+	+	+

+ An obvious red band was shown

coating colloidal surfaces with protein molecules such as immunoglobulin. Properties of the individual antibody can extremely affect the preparation of individually modified antigen-specific antibody–gold conjugates.

In the present study, an IC strip test was developed successfully with a colloidal gold–digoxin-specific mAb conjugate. This IC test strip is an easy, fast, quantitative, and competitive binding immunoassay for the determination of digoxin in serum at or above 2 ng/ml. The method employs a unique monoclonal antibody to selectively identify digoxin in test samples with a high degree of sensitivity and specificity for the monitoring of digoxin in serum of digitalized patients. A highly sensitive chromatographic system is desirable for monitoring digoxin because it has a narrow therapeutic range (0.8–1.9 ng/ml).

In this assay, 100 μl of standard or sample was applied to the sample pad and then allowed to migrate up the membrane.

As the reaction mixture reaches the test line containing Dig–BSA, a competition occurs between the free digoxin in the sample and the solid-phase Dig–BSA for binding to anti-Dig probe. When the solution reaches the zone immobilized with goat anti-mouse antibodies, any non-specific interaction can be observed in the sample. The positive result is judged by the appearance of one red line in the control region. The negative result is judged by the appearance of two red lines in control and test regions. The color signal can be read within 5 min, and the intensity determines the amount of the analyte. In this study, to develop a sensitive IC test strip, different volumes of gold nanoparticle-conjugated antibody and also different concentrations of Dig–BSA were examined. When we used 2 μg/μl of Dig–BSA and 0.7 μl/strip of colloidal gold mAb, we could determine digoxin in serum equal or above 2 ng/ml.

To determine the detection limit of the test strip, we tested a series of dilutions of digoxin standard in normal serum (0–3 ng/ml) by the IC strip test and results are shown in Fig. 7. We found that, whereas a weak red line in the result line was shown at 1.8 ng/ml of digoxin, no color signal in the result line was observed at ≥2 ng/ml. Therefore, in order to decrease false positive reactions in a non-toxic serum, we optimized the detection limit at 2 ng/ml. The specificity of IC test strip was evaluated with digoxin, digoxin-like compounds, and other steroids. The result shown in Table 1 indicates that the developed IC test strip has high specificity to digoxin and no reaction is observed with other compounds. The reliability of the test was examined by carrying out the IC strip test with 40 serum samples obtained from digitalized patients and the results of these tests were compared with those obtained by RIA. The results demonstrated that two methods have excellent correlation in the diagnostic judgment and the sensitivity of this test strip is 100%, the specificity is 96%, and the positive predictive value (PPV) is 93% with the negative predictive value of 95%. For the quantitative measurement of digoxin in serum, a colloidal gold IC test strip has been reported [20], but the sensitivity of that (detection limit 1 μg/ml) was lower than the IC test strip in this research. Therefore, a more sensitive method may be necessary in emergency conditions for detecting the level of digoxin toxicity. In conclusion, the IC test strip we developed in the present study showed a high sensitivity and the detection limit of the IC test strip was 2 ng/ml.

In summary, according to its speed and simplicity, the IC test strip assay is generally preferred to other immunoassays such as RIA and ELISA. Furthermore, it has a potential application for home tests, emergency cases, and use in the absence of advanced instrumentation.

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