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Immunochromatographic paper sensor for ultrasensitive colorimetric detection of cadmium

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ABSTRACT

A novel and highly sensitive immunochromatographic strip based on a monoclonal antibody (3A9) was developed for the detection of cadmium in tap water. The 50% inhibition concentration of the antibody, which showed no cross-reactivity with other heavy metal ions, was 0.45 ng/mL and it recognized Cd(II)–1-(4-isothiocyanobenzyl) ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA) (Cd(II)–ITCBE) and not metal-free ITCBE and EDTA. The cutoff value for semi-quantitative detection of the strip was 5 ng/mL and the lower limit of detection for quantitative detection was 0.2 ng/mL using a scanning reader. The percent recovery ranged from 107.6% to 132% in tap water samples.

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Introduction

Cadmium (Cd) is one of the most highly toxic heavy metals which is non-biodegradable and has a long half-life in the biosphere (Singh, Sharma, Agrawal, & Marshall, 2010). The main sources of cadmium pollution are non-ferrous metal smelting, effluents released from electroplating and fertilizer (Mclaughlin & Singh, 1999). Cd has deleterious effects on human health through enrichment of the alimentary chain (Rajaganapathy, Xavier, Sreekumar, & Mandal, 2011). Some studies have suggested that Cd exposure may lead to diabetes (Edwards & Prozialeck, 2009), lung cancer (Persico et al., 2006), hypertension, osteoporosis (Jin et al., 2004) and other diseases. The World Health Organization and Bureau of Indian Standards provide values for the toxicity range of cadmium that are as low as 0.005 mg/L and 0.01 mg/mL, respectively (Ercal, Gurer-Orhan, & Aykin-Burns, 2001). The requirement for safe drinking water makes fabricating a convenient, inexpensive and rapid method to monitor Cd in the environment an urgent necessity.

At present, inductively coupled plasma–mass spectroscopy (ICP–MS) (Beiraghi, Pourghazi, & AmoliDiva, 2014), flameless atomic absorption spectrometry (Prabhakaran, Yuehong, Nanjo, & Matsunaga, 2007) and atomic fluorescence spectroscopy (Sánchez-Rodas, Corns, Chen, & Stockwell, 2010) are used to detect cadmium. Undeniably, these

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instrumental analytical methods are precise, sensitive and specific, but they have plenty of restrictions; for example they are expensive, require a professional analyst, take a long time and are laboratory bound (Gumpu, Sethuraman, Krishnan, & Rayappan, 2015). To overcome these shortcomings, researchers have developed biosensors to detect heavy metals in recent years. These biosensors are composed of a range of materials such as metal-specific DNAzymes (Fang et al., 2010; Hao, Xua, Xing, & Kuang, 2012; Mazumdar, Liu, Lu, Zhou, & Lu, 2010; Yin et al., 2014), Raman label-encoded gold-nanoparticles (GNPs) (Li et al., 2015; Ma et al., 2013), carbon nanoparticles (Aragay, Pons, & Merkoçi, 2011), chiroplasmonic assemblies (Xu et al., 2013), metal-specific monoclonal antibodies (Blake et al., 2001) and nucleic acid functionalized gold nanorods (Xu, Kuang, Wang, & Xu, 2011; Zhu et al., 2012).

The immunochromatographic assay, which is cheap, time-saving, specific and sensitive based on an antibody, is widely used for the rapid detection of cadmium. Zhan et al. developed a reliable enzyme-linked immunosorbent assay (ELISA) for detection of cadmium using a polyclonal antibody prepared through injecting BALB/c mice with Cd–2-(4-aminobenzyl)–diethylene triamine pentaacetic acid–keyhole limpet hemocyanin (Cd–DTPA–KLH). The polyclonal antibody whose 50% inhibition concentration (IC_{50}) value was 2.042 μg/mL can specifically recognize Cd (Zhan, Xi, & Zhou, 2015). Xing et al. prepared an immunochromatographic strip using a monoclonal antibody specific for Cd–ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA). Through silver enhancement, the visual detection limit for Cd–EDTA of this strip was 5 ng/mL (Xing, Kuang, et al., 2014). Adaris et al. produced a novel lateral flow immunoassay device (LFID) based on competitive reaction between the Cd–EDTA–bovine serum albumin–gold-nanoparticle (Cd–EDTA–BSA–GNP) conjugate and the Cd–EDTA complex to the immobilized 2A8 monoclonal antibody in the test line. The quantification limit of the LFID was 0.4 ng/mL. The LFID was sensitive but it relied on availability of a scanning reader (López_Marzo, Pons, Blake, & Merkoçi, 2013).

This paper introduces a highly sensitive immunochromatographic strip based on the anti-Cd–1-(4-isothiocyanobenzyl) EDTA (ITCBE) monoclonal antibody for Cd detection. It has come to our knowledge that it represents the most sensitive immunochromatographic strip, which can be evaluated visually. For good measure, we also used it to detect the residual amount of Cd in tap water.

Materials and methods

Materials

Cu(II), Hg(II), Cd(II), Pb(II), Mn(II), Zn(II), Al(III), Mg(II) and Ca(II) (1 mg/mL in 1 M HNO_3) were purchased from the National Institute of Metrology, P.R. China (Beijing, China) and were used as the atomic absorption standards. KLH, BSA, incomplete Freund's adjuvant (FIA), complete Freund's adjuvant, 3,3′,5,5′-tetramethyl-benzidine, Tween-20, horseradish peroxidase (HRP) and gelatin goat anti-mouse immunoglobulin G (IgG) and chloroauric acid ($HAuCl_4 \bullet 4H_2O$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The ITCBE was obtained from Dojindo Laboratories (Kumamoto, Japan). Cell culture media, including polyethylene glycol (PEG) solution, Hypoxanthine-aminopterin-thymidine monophosphate (HAT) supplement, HT

supplement and RPMI 1640 cell culture medium, were obtained from Life Technologies (Thermo Fisher Scientific, Waltham, MA, USA). Mouse SP2/0 myeloma cells were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). All water used was purified to 18.2 MΩ cm, and 10 mM HBS:(HEPES Buffer Solution), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer sollution containing 2.383g HEPES, 8.775g NaCl and 0.2g KCl in 1 L water. The solution is adjusted to pH 9 using KOH.

The backing material (polyvinylchloride), sample pad (glass fiber membrane, GL-b 01) and absorbance pad (H5079) were supplied by JieYi Biotechnology Co. Ltd. (Shanghai, China). The nitrocellulose (NC) membrane was purchased from Merck-Millipore (Darmstadt, Germany).

Production of anti-Cd(II)–ITCBE monoclonal antibody

We prepared complete antigens using the bifunctional chelator ITCBE connecting metal ions and proteins (Xing, Hao, Liu, Xu, & Kuang, 2014). One milligram ITCBE dissolved in 0.1 mL dimethyl sulfoxide and 5 mg KLH were added to 5 mL 10 mM HBS (pH 9.0) and stirred overnight at room temperature. Next day the solution was added drop-wise together with 0.6 mL Cd standard solution (1 mg/mL in 1 M HNO_3) to the solution, keeping the pH at 8.0, and stirred for 3 h at room temperature. Finally the unreacted small molecules were removed by ultrafiltration at 6200*g* for 30 min. Cd–ITCBE–KLH as immunogen was stored at −20°C. Cd–ITCBE–BSA was prepared using the same process as antigen coating.

Five female BALB/c mice were injected subcutaneously with the mixture of Freund's adjuvant and 100 μg immunogen for the first immunization. A mixture of FIA and 50 μg immunogen per mouse was injected in the booster immunizations. After the fourth injection, an indirect competitive ELISA (ic-ELISA) was used to analyze the serum. The mouse whose splenocytes produced the most sensitive antibody against Cd(II)–ITCBE was selected to fuse with Sp 2/0 myeloma cells to produce hybridoma cells. After cell screening, 3A9, which produced the most sensitive antibody against Cd(II)–ITCBE but not ITCBE or EDTA, was injected into mouse peritoneum to produce ascetic fluid (Deng et al., 2012). The ammonium-saturated ammonium sulfate precipitation method was used to purify the antibody. We calculated the sensitivity and cross-reactivity of the antibody by ic-ELISA. The IC_{50} of the antibody produced by 3A9 was 0.45 ng/mL.

Preparation of the immunochromatographic assay

Preparation of GNPs

GNPs of 20 nm diameter were synthesized by citric acid reductant (Saha, Agasti, Kim, Li, & Rotello, 2012). Before starting the synthesis, all glassware used was thoroughly soaked in aqua regia (HCl/HNO_3, volume ratio 3:1) overnight, rinsed with Millipore-Q water and dried. First, 500 mL of 0.01% $HAuCl_4$ solution was boiled thoroughly with continuous stirring, then 10 mL of 1% trisodium citrate solution was added and the mixture was boiled for additional 10 min. The solution was cooled to room temperature and stored at 4°C for antibody labeling. The GNPs were distributed evenly and the diameter of 20 nm was confirmed by transmission electron microscopy (TEM) (Figure 1).

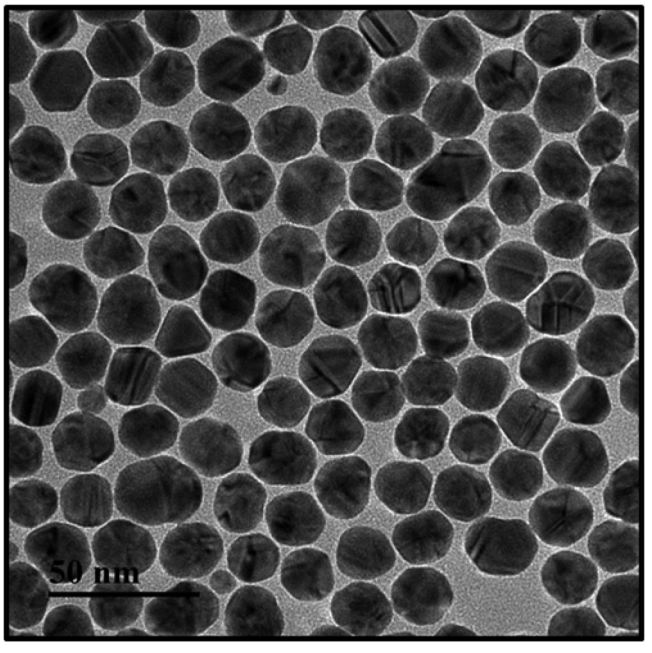

Figure 1. TEM image of the GNPs with the inset graph that shows the nanoparticles distribution size.

Preparation of antibody–GNP conjugate

The pH of 10 mL colloidal gold solution was adjusted to 8.2 with 0.1 M K_2CO_3, then 1 mL anti-Cd(II)–ITCBE monoclonal antibody (0.2 mg/mL in 2 mM borate buffer) was added. After 30 min mixed reaction, the gold particles were blocked with 0.5 mL 10% BSA solution for 2 h and then centrifuged at 6200*g* for 30 min. The resulting precipitate was resuspended in 1 mL resuspension buffer composed of 10 mM Tris/HCl buffer (pH 8.2), 0.5% (w/v) BSA, 0.5% (w/v) PEG and 0.01% (w/v) NaN_3 and stored at 4°C (Peng et al., 2016).

Optimization of the strip

Many factors such as pH, the amount of antibody and coating antigen affect the determination of the strip; so we optimized them to increase the sensitivity (Kuang et al., 2013). The pH of the GNP solution was adjusted from 7.0 to 9.0 using 0.1 M K_2CO_3. The concentration of anti-Cd(II)–ITCBE antibody in the reacting solution and the coating antigen on the NC membrane were optimized from 25 to 8 μg/mL and 0.5 to 0.05 μg/mL, respectively. Finally the optimal conditions were found to be pH 8.2, 20 μg/mL anti-Cd(II)–ITCBE antibody and 0.1 μg/mL coating antigen and these were used to prepare the strip.

Assembly of immunochromatographic strip

The strip was assembled from an NC membrane, a sample pad and an absorbent pad as shown in Figure 2. The Cd(II)–ITCBE–BSA (0.1 mg/mL) and goat anti-mouse IgG (0.5 mg/mL) were sprayed onto the NC membrane as the test line (T line) and the

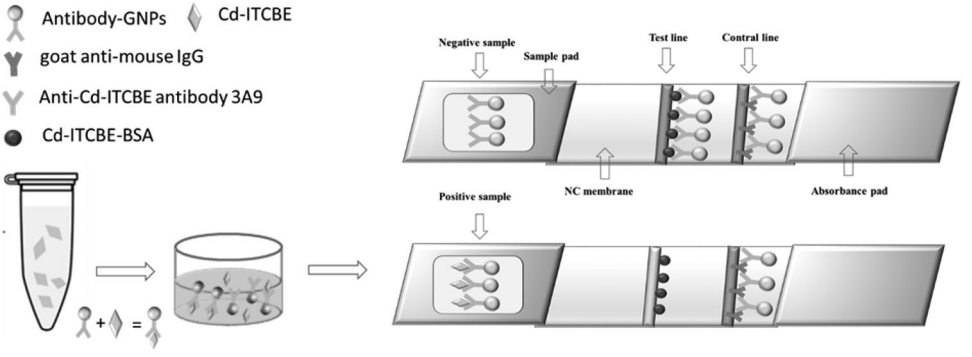

Figure 2. The schematic illustration of strip.

control line (C line), respectively, using a dispenser at 1 μL/cm. The strip was then dried at 37°C overnight, then the absorbent pad and sample pad were laminated to the strip and the resulting assembly was cut into 2.8 mm width strips. Then 5 μL concentrated GNP–antibody conjugate and 45 μL resuspension buffer was added into every well of a 96-well plate and freeze-dried.

Immunochromatography for the detection of cadmium

To calculate the linear range and the limit of detection (LOD), a series of Cd–ITCBE complex standard solutions was prepared with 10 mM HBS (pH 7.4) and ITCBE (0.1 mM) containing Cd concentrations of 10, 5, 2, 1, 0.5 and 0.2 ng/mL. Next, 200 μL Cd–ITCBE complex was added to the freeze-dried GNP–antibody, stirred evenly and incubated for 3 min at room temperature. Then the strip was inserted into the micropore with the sample pad down and allowed to react for 5 min. After removing the test strip the result was interpreted within 3 min. The LOD concentration was the cutoff value when there was only a visible control line but no observable test line on the strip. For quantitative determination, the chromaticity of each strip was recorded using a strip reader and the LOD was calculated.

Water sample analysis

Water samples were spiked with specific concentrations of Cd(II) and the pH was adjusted to 7.4 with 1 M NaOH. Next a 10-fold concentrated sample treatment solution containing 100 mM HBS (1.37 M NaCl, 30 mM KCl and 100 mM HEPES, pH 7.4) and 1 mM ITCBE was added to the samples. The spiked and unspiked samples were analyzed three times using the strip.

Results and discussion

Characterization of the sensitivity and specificity of the antibody

The cell line (3A9) which produced the anti-Cd(II)–ITCBE monoclonal antibody was selected after three rounds of subcloning. The sensitivity and specificity of the monoclonal

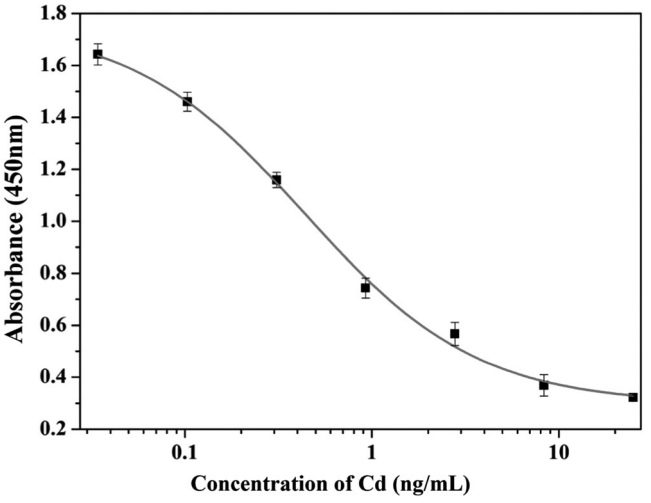

Figure 3. Standard curve obtained by ic-ELISA based on antibody 3A9.

antibody obtained by the purification of the ascites were tested by ic-ELISA. The IC_{50} was 0.45 ng/mL and the linear range was 0.15–18 ng/mL as shown by the typical inhibition curve shown in Figure 3. Nine other metal ions, including Cu(II), Pb(II), Mn(II), Al(III), Mg(II), Hg(II), Zn(II), Cr(III) and Ca(II), were also tested using the ic-ELISA. As shown in Table 1, the cross-reactivity of the antibody to these nine other metal ions was less than 0.2%. Thus the monoclonal antibody obtained was very specific to Cd(II)–ITCBE, and any other metal ions present did not affect the detection of Cd in tap water.

Operation principle and evaluation of the immunochromatographic assay

The principle of the immunochromatographic assay was based on the competitive binding of Cd–ITCBE–BSA immobilized in the test zone and Cd–ITCBE complex in the sample to the anti-Cd–ITCBE antibody. The operation of the immunochromatographic assay is shown in Figure 2. To perform the test, 200 µL standard solution was added to the micropore containing the freeze-dried GNP–antibody. If there was no Cd(II) in the sample, the antibody–GNP complex would combine with the immobilized antigen, causing the test zone to appear red, indicating that the sample was

Table 1. Cross-reactivity of nine other metal ions.

Metal ions	IC_{50} (ng/mL)	Cross-reactivity (%)
Cd(II)	0.45	100
Cu(II)	>500	<0.1
Ca(II)	>500	<0.1
Zn(II)	>500	<0.1
Mn(II)	>100	<0.5
Mg(II)	>500	<0.1
Al(III)	>500	<0.1
Pb(II)	>500	<0.1
Ni(II)	>500	<0.1
Hg(II)	>500	<0.1

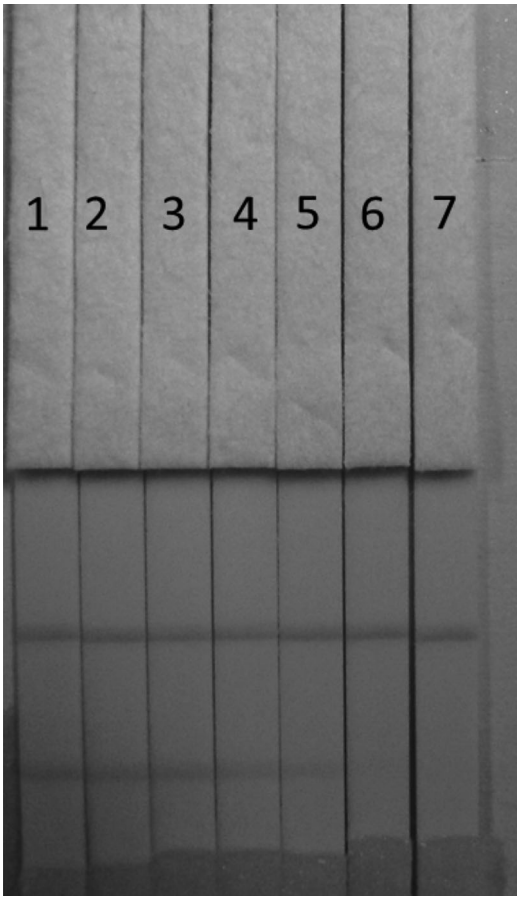

Figure 4. Typical photo image of detection of Cd(II) by strip sensors in sample treatment solution (0, 0.2, 0.5, 1, 2, 5, 10 ng/mL from left to right).

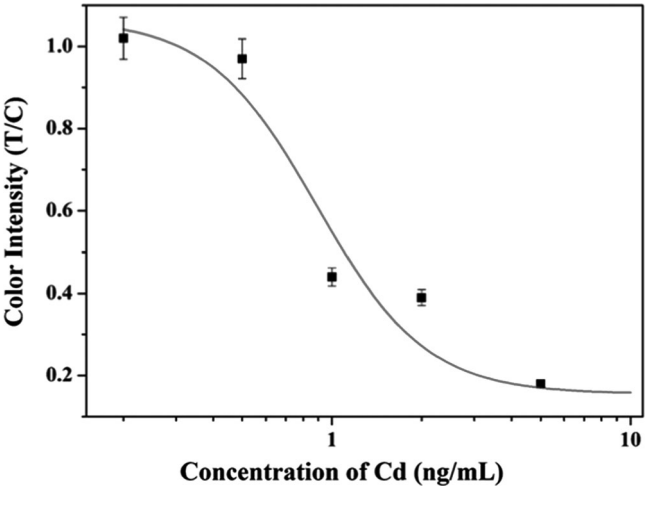

Figure 5. Linear range of the calibration curve obtained during Cd(II)–ITCBE analysis by strip immunoassay.

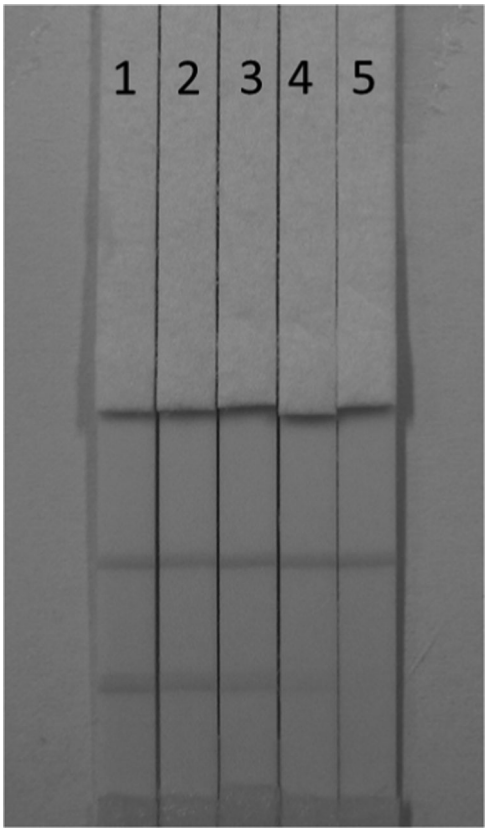

Figure 6. Typical photo image of detection of Cd(II) spiked with a series of Cd concentration (0.2, 0.5, 1, 2, 5 ng/mL from left to right) by strip sensors in tap water.

negative. With the increase of Cd(II) concentration, more of the antibody–GNP complex would bind to the Cd–ITCBE complex resulting in the red of the test zone becoming weaker until it disappeared.

A series of Cd–ITCBE standard solutions in which the concentrations of cadmium were 10, 5, 2, 1, 0.5, 0.2 and 0 ng/mL were tested by the method (Figure 4). When the concentration of cadmium was 5 ng/mL, the red color disappeared. The chromaticity of each strip was recorded with a strip reader, and the LOD was calculated from the calibration curve shown in Figure 5.

Table 2. Recovery test of Cd(II) in tap water samples.

Spiked concentration (ng/mL)	Test strip visual color intensity	Scanning reader detected concentration (mean ± SD, $n = 3$) (ng/mL)	Recovery (%)	Coefficient of variation (CV) (%)
0.2	+++	0.24 ± 0.03	115	12.1
0.5	+++	0.67 ± 0.04	132	11
1	+++	1.05 ± 0.08	104	9.7
2	±±±	2.39 ± 0.06	119	1.7
5	−−−	5.39 ± 0.07	107.6	1.3

Note: +, presence of Cd(II) in sample; ±, weakly positive; −, absence of Cd(II).
[a]Original concentration was 0.01 ng/mL detected by ICP–MS.

Detection of Cd(II) ion in water samples

Water samples were spiked with specific concentrations (0.2, 0.5, 1, 2, 5 ng/mL) of Cd(II) and the pH was adjusted to 7.4 with 1 M NaOH. Subsequently a 10-fold concentrated sample treatment solution was added to the samples, and allowed to react for 10 min. The spiked and unspiked samples were analyzed three times using the strip. When the concentration of Cd(II) was above 5 ng/mL, the test line disappeared, consistent with the result in Cd–ITCBE complex standard solution (Figure 6). The percent recovery (107.6–132%) was obtained from the calibration curve as shown in Table 2.

Conclusion

In this study, we developed an ultrasensitive and specific immunochromatographic strip for the detection of Cd in tap water. The cutoff level was 5 ng/mL, which could be used as the visual LOD for semi-quantitative assay while the LOD for quantitative evaluation was 0.2 ng/mL using a scanning reader. The percent recovery ranged from 107.6% to 132% in tap water samples and the total detection time was only 10 min. In summary the assay was sensitive, specific, did not require a specialist operator, low cost and time-saving; so it could be employed as a rapid screening tool for the detection of Cd on the spot.

Disclosure statement

No potential conflict of interest was reported by the authors.

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