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Colloidal gold nanoparticle probe-based immunochromatographic assay for the rapid detection of chromium ions in water and serum samples

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Abstract

An immunochromatographic assay (ICA) using gold nanoparticles coated with monoclonal antibody (McAb) for the detection of chromium ions (Cr) in water and serum samples was developed, optimized and validated. Gold nanoparticles coated with affinity-purified monoclonal antibodies against isothiocyanobenzyl-EDTA (iEDTA)-chelated Cr³⁺ were used as the detecting reagent in this competitive immunoassay-based one-step test strip. The ICA was investigated to measure chromium speciation (Cr³⁺ and Cr⁶⁺ ions) in water samples. Chromium standard samples of 0-80 ng/mL in water were determined by the test strips. The results showed that the visual lowest detection limit (LDL) of the test strip was 50.0 ng/mL. A portable colorimetric lateral flow reader was used for the quantification of Cr. The results indicated that the linear range of the ICA with colorimetric detection was 5-80 ng/mL. The ICA was also validated for the detection of chromium ions in serum samples. The test trips showed high stability in that they could be stored at 37°C for at least 12 weeks without significant loss of activity. The test strip also showed good selectivity for Cr detection with negligible interference from other heavy metals. Because of its low cost and short testing time (within 5 min), the test strip is especially suitable for on-site large-scale screening of Cr-polluted water samples, biomonitoring of Cr exposure, and many other field applications.

Keywords

Chromium ions; Gold nanoparticle; Immunochromatography assay; Rapid test; Quantification

Introduction

With the increasing industrial uses, many heavy metals enter into subsistent wastes through various routes and cause direct or indirect harm to the environment and humans. Heavy metals, such as copper, lead, zinc, iron, cobalt, nickel, chromium, cadmium, mercury,

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tungsten, molybdenum, gold and silver are not biodegradable. Biological uptake of heavy metals from the environment through the food chain leads to tens of thousands of times enrichment in higher organisms, and human consumption through the food chain could cause chronic poisoning. Although heavy metals, such as chromium (Cr), manganese, copper, zinc and others are trace elements required for life activities, the same elements could be toxic to humans beyond certain concentrations[1, 2].

High Cr absorptions in vivo can result in various diseases, such as fibroproliferative disease, airway hypersensitivity, lung cancer, nasal cancer, and other types of cancer. In addition to adduct formation, exposure to Cr can cause various point mutations in DNA, chromosomal damage, and oxidative changes in proteins[3, 4]. The health effects of different forms of Cr are different because each form has different toxicity. Cr^{6+} is classified as a carcinogen by the National Toxicology Program, while Cr^{3+} is much less toxic without carcinogenesis toxicity. The U.S. Environmental Protection Agency (EPA), World Health Organization (WHO), and European Community (EC) have set chromium limits of 100 $\mu\text{g/L}$ (EPA 822-R-06-013), 50 $\mu\text{g/L}$ (WHO ISBN: 9241546743), and 50 $\mu\text{g/L}$ (98/83/EC), respectively, in drinking water. The content of Cr^{3+} in serum could be detected to diagnose chromium poisoning or deficiency. Serum chromium levels normally range from less than 50 up to 500 ng/mL. Normal value ranges may vary slightly among different laboratories[5]. It is also known that Cr can easily change from one form to another in water and soil, depending on the conditions present. Therefore, Cr^{6+} could be reduced to Cr^{3+} easily through pretreatment.

A number of methods have been reported for the detection of metal ion, such as dispersive liquid-liquid microextraction and dried-droplet laser ablation ICP-MS[6], carbon composite-PVC based membrane coated platinum electrode[4], amperometric enzyme-based sensor[7], flame atomic absorption spectrometry[8], fluorine-doped graphite pencil electrode[9] and nano-Au/TiO₂ photocatalysis reduction device[10]. Despite the high sensitivity and specificity that can be achieved by these methods, they require professional staff and expensive equipment that are difficult to use on-site. In contrast to instrumental method, an immunochromatography test strip has its unique advantages, such as simple and low-cost instrumentation requirements, simple operation, and fast results obtained within 3-5 min[11]. So far, most of test strip based assays focus on semi-quantitative analysis based on visual observation. The low sensitivity of the test strip based assays limit their applications for environmental monitoring and biomedical analysis. Recently, nanoparticle probes combined with a fluorescent reader or electrochemical reader have been developed for enhanced sensitivity for detection of biomarkers using the test strip assays[12-14].

In this study, an integrated test strip based device with Au nanoparticle probe combined with a portable colorimetric lateral flow reader was developed for the quantification of Cr. The device was utilized to measure Cr speciation (Cr^{3+} and Cr^{6+}) in water samples. The test strip based assay was also validated with the detection of Cr ions in serum samples. The results demonstrated the feasibility, sensitivity and specificity of this gold nanoparticle probe-based immunochromatographic assay, indicating it could be used for rapid detection of Cr ions in water samples, serum samples and other field applications.

Materials and methods

Chemical reagents, apparatus, and other materials

Chromium ion foil (99.999 %) was obtained from Aldrich Chemical (Milwaukee, WI, USA). Other metals in salt form, including Fe^{3+} , Pb^{2+} , Hg^{2+} , Cu^{2+} , Al^{3+} , Mg^{2+} , Ca^{2+} , Zn^{2+} and Co^{2+} were purchased from Merck Chemical (Darmstadt, Germany). Isothiocyanobenzyl-EDTA (iEDTA) was purchased from Dojindo Laboratories (Kumamoto,

Japan). Bovine serum albumin (BSA, molecular weight (MW) 67,000), goat anti-mouse immunoglobulin G (IgG), chloroauric acid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$), polyethylene glycol (PEG, MW, 2000), polyvinylpyrrolidone (PVP, MW 40000) and S-17 (Rhodasurf ON-870) were obtained from Sigma (St Louis, MO, USA). Ascites containing anti-Cr-EDTA monoclonal antibody, Tris-HCl buffer and Phosphate Buffered Saline (PBS) were prepared in our laboratory. Ultrapure water was produced using the Milli-Q Ultra Pure System (Millipore, Bedford, MA, USA). BCA protein assay kit was purchased from Pierce (Rockford, IL, USA). All chemicals used were analytical grade or higher. All glassware and plastic wares were pretreated with 6.0 M hydrochloric acid solution for 1 h and then transferred into 7.2 M nitric acid solution for 1 h, then rinsed thoroughly with ultrapure water before use. Nitrocellulose (NC) membranes (HFB13504) were purchased from Millipore (Shanghai, China). PVC sheets, adhesive tape and filter paper were purchased from local market (Shanghai, China).

Other equipments used in this study include a 4000 Q TRAP LC/MS system (Applied Biosystems, Framingham, MA, USA), transmission electron microscope (TEM, PHILIPS TECNAI, Holland), ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA), ultraviolet-visible spectrophotometer (Shimadzu, Japan), the Milli-Q Ultra Pure System (Millipore, Bedford, MA, USA), magnetic stirrer (Beike, Beijing), centrifuge (Beckman, Germany), and microplate reader (Thermo Fisher Scientific). The platform consists of motion control with Biostrip Dispenser HGS102, Airjet HGS102, and the programmable strip cutter HGS201 (purchased locally in Shanghai, China). The AESE-Quant Portable and Benchtop Lateral Flow Reader was purchased from DCN Inc. (Irvine, CA).

Preparation of the coating antigen

The low-molecular-weight Cr was too small to elicit an immune response. Therefore, iEDTA, a highly effective bifunctional chelating agent, was selected to conjugate Cr and carrier proteins (Fig. 1). EDTA-protein complexes were prepared by an appropriate modification method[15]. 0.4mg of iEDTA was dissolved in 100 mL of dimethyl sulfoxide (DMSO), and 3.4 mg of BSA was dissolved in 1.0 mL Tris-HCl buffer (0.1 M, pH 8.5). The reaction mixtures were adjusted to pH 9.0 with triethylamine and stirred for 24 hours at 25°C. Small unconjugated molecules were removed by buffer exchange with centricon-30 micro-concentrators. The conjugates were washed with metal-free Tris-HCl buffer (0.1 M, pH 7.4), and then 80 μL of chromic chloride (1 M) was added dropwise with gentle stirring to the purified EDTA-protein conjugate while stirring gently. The complex reaction was allowed to proceed for 4 h at 25°C. Finally, the unreacted Cr ions were removed as described. The concentration of protein conjugate was determined by Pierce BCA protein assay. The amount of Cr of the complete antigens was detected by inductively coupled plasma mass spectroscopy (ICP-AES)[16].

Hybridoma production and antibody purification

Four 6-week-old female BALB/C mice were immunized with Cr^{3+} -iEDTA-BSA on days 0, 14, and 28. Three cell lines secreting monoclonal antibodies against Cr^{3+} -iEDTA were established by using the cell fusion technology, indirect ELISA and competitive ELISA. Hybridomas synthesizing and secreting metal-specific antibodies were subcloned twice by limiting dilution. Positive hybridoma clones were subcultured and frozen. The best cell line coded as 5G₁₂C₅ was selected to produce ascitic antibodies by injecting 10⁶ hybridoma cells in BALB/c mice.

The mouse ascitic antibodies were precipitated with saturated ammonium sulfate and dissolved in phosphate buffer (0.002 M, pH 7.4) and then were dialyzed against the same buffer at 4°C for 2 days. The IgG proteins were isolated by affinity chromatography with an

immobilized protein G column. The antibody concentration was determined by using the BCA protein assay.

Preparation of gold nanoparticles

Colloidal gold nanoparticles with a mean particle diameter of 20 nm were produced by reduction of gold chloride with 1% sodium citrate[17]. Briefly, 100 mL of 0.02 % gold chloride trihydrate solution in superpurified water was heated to boiling, and then 4.0 mL of 1 % sodium citrate solution was added quickly into the solution under stirring over a period of 20 min. Gradually, the color changed from light yellow to brilliant red. After the color change, the solution was boiled for another 20 min to complete the reduction of gold chloride, cooled and stored at room temperature with 0.05% sodium azide.

Conjugation and purification of McAb-gold complexes

A preliminary titration was performed to determine the optimum conditions for conjugation between gold and the antibody. The pH of colloidal gold solution was adjusted to 8.5 with 0.25 M K₂CO₃. The optimum protein concentration for labeling was determined by the following two steps: (1) Ascetic anti-Cr-EDTA McAbs were diluted with PBS (0.015 M, pH 7.4) into gradient concentration and 10 μ L solution of each concentration was added into 100 μ L of colloidal gold solution. (2) After incubation for 15 min at room temperature, 20 μ L 20% NaCl was added into the solution. The color of the reaction changed from brilliant red to blue as the concentration of McAbs decreases. The lowest concentration of McAbs solution to prevent the color change was determined as the minimum concentration for gold labeling. The color change was measured by light absorbance at 570nm using a microplate reader. The amount of anti-Cr-EDTA McAbs used for gold conjugation was in excess by 30%.

According to the minimum concentration of McAbs for gold labeling, 0.02 mL of purified anti-Cr-EDTA McAbs (2 mg/mL) was diluted to 100 μ L with distilled water and added dropwise to 100 mL pH-adjusted colloidal gold solution. After incubation for 30 min, the mixture was added with 11 mL of 10% BSA drop by drop, incubated for 30 min, and finally centrifuged at 10,000 rpm for 15 min to remove the unconjugated antibodies. After removal of the supernatant, precipitate was resuspended with PBS (0.015 M, pH 7.4) containing 5% (w/v) sucrose, 1% (w/v) BSA and 0.02% (w/v) sodium azide and stored at 4°C for further use. The conjugation was confirmed by UV-Vis spectroscopy.

For the detection of the serum samples, the added volume of the purified anti-Cr-EDTA McAbs for labeling was 0.08 mL; the other steps were the same as the above mentioned.

Preparation of the immunochromatographic strips

Immunochromatographic test strips were assembled using the method described previously [18]. The gold-labeled anti-Cr-EDTA McAb was dispensed by Airjet HGS102 onto the glass fiber pad. Cr-EDTA-BSA was diluted with PBS (0.01 M, pH 7.4) containing 5% trehalose for antigen protection when the NC membrane was dried at 37°C[19]. The Cr-EDTA-BSA was dispensed at the lower side of the NC membrane to form the test (T) line, while 2 mg/mL goat anti-mouse IgG was dispensed at the upper side to form the control (C) line. The distance between the T and C line was about 5 mm. Because the sensitivity of the test strip should be adjusted to detect different samples, the concentrations of Cr-EDTA-BSA were diluted to 140-560 ng/mL.

The sample pad was treated with PBS (0.15 M, pH 7.4) containing 0.5% BSA to remove non-specific reactivity on the NC membranes[11]. It was further treated with 1% trehalose, 0.02% sodium azide, and 2% S-17 (Rhodasurf ON-870) and dried at 37°C before use, while

the absorbent pad was applied immediately without treatment. The PVC plate served as the plastic support of the test strip. The NC membrane, conjugate pad, sample pad, and absorbent pad were laminated and pasted onto the PVC plate, which was then cut into 4-mm-wide and 60-mm-long strips using the programmable strip cutter HGS201. All strips were sealed in a plastic bag with a pack of desiccant gel and stored at 4°C. The cross-section of an immunochromatographic strip is shown in Fig. 2.

Determination of hexavalent and total chromium

Because Cr^{6+} cannot be chelated with EDTA, the prepared monoclonal antibody was designed to target against Cr^{3+} -EDTA. To measure Cr^{6+} consistently, the pH values of samples were adjusted to 8.0-9.0 to precipitate Cr^{3+} . Then 1 mL of the collected supernatant containing Cr^{6+} was reduced to Cr^{3+} by 1 mL of reducing agent sodium bisulfite (NaHSO_3 , 60 mg/L) in the presence of 0.5 mM EDTA, which was used to chelate the formed Cr^{3+} , and the pH was subsequently adjusted to 7.4. The reduction reaction is represented by the following equation:

Finally the Cr^{3+} concentration of the reduced solution was determined by ICA, which is equal to the Cr^{6+} concentration of the original sample.

To determine the total Cr, the pH values of samples were adjusted to 3.0 to avoid Cr^{3+} precipitation. 1 mL of 60 mg/L NaHSO_3 containing 0.5 mM EDTA was added, and the pH of sample water was adjusted to 7.4, followed by Cr determination with ICA. The Cr^{6+} and total chromium contents in all water specimens were also measured by ICP-AES.

To verify the potential of quantitative detection, the applied strips were read by AESE-Quant portable and benchtop lateral flow reader.

Detection of the chromium ions in water samples

Water samples for Cr ion detection were collected from the Pearl River (Guangzhou, China) and the West Lake (Huizhou, China). Both water samples were pretreated and incubated at room temperature. Cr ions concentrations were finally measured with ICA.

Detection of the chromium ions in human serum samples

Human serum samples were pretreated by isopyknic 1M HNO_3 to isolate Cr^{3+} from proteins[20]. The reaction mixtures were adjusted to pH 6.5 with 1M NaOH. Then 0.5 mM EDTA was added to chelate Cr^{3+} . The pretreated serum samples were measured with ICA.

Cross-reactivity of the test strip

To evaluate the specificity of the test strip, the EDTA complexes of many metal ions, including Fe^{3+} , Pb^{2+} , Hg^{2+} , Cu^{2+} , Al^{3+} , Mg^{2+} , Ca^{2+} , Zn^{2+} and Co^{2+} were prepared and tested at a concentration gradient.

Results and Discussion

Characterization of Cr-EDTA-BSA

The mean Cr concentration of the complete antigen Cr-EDTA-BSA was 28.0 $\mu\text{g/mL}$ as measured by ICP-AES. The results demonstrated that Cr^{3+} had been coupled to carrier proteins successfully through iEDTA, a highly efficient bifunctional metal chelating agent.

Characterization of the gold nanoparticles

Gold nanoparticles were used as a probe in immunochromatographic detection because of the easy preparation of required size, monodispersity in aqueous phase and application as detector reagents by conjugation with biomolecules[21]. To measure the size and size distribution of these particles, the gold nanoparticles were scanned under a TEM, and the results showed that the average diameter of these particles was approximately 20nm with a good size distribution (Fig. 3).

Characterization of the gold nanoparticles coated with antibodies

Gold-protein complexes, in general, are formed by electrostatic attraction between the negatively charged gold particle and the positively charged protein molecules[22]. Sometimes the Fab region of an antibody may conjugate to colloidal gold, which makes the antibody unable to react with the antigen[23].

Colloidal gold nanoparticles were scanned with UV-Vis spectroscopy from 420 to 700 nm[24]. There was only one maximum absorbance wavelength at 520 nm, which is the characteristic absorbance peak of colloidal gold. To make sure that the McAbs had been absorbed on the colloidal gold successfully, colloidal gold-labeled anti-Cr-EDTA McAbs were scanned by UV-Vis spectroscopy. The results demonstrated that the maximum absorption wavelength had a red shift compared with that of the naked colloidal gold nanoparticles (Fig. 4).

Assay principle and sensitivity of the test strip

When the test strips were applied with liquid samples, the McAb-gold complexes could be dissolved and flow forward with the sample solution. If there was no Cr^{3+} in sample solution, the McAb-gold complexes could be captured by antigen and goat anti-mouse IgG, which were dispensed at the T line and C line. The strip will show two red lines with close color density. If there was Cr^{3+} in the sample solution, the McAb-gold complexes would react with the Cr^{3+} in positive sample solution, which results in fewer McAb-gold complexes to be bound with antigen located at the T line. Therefore, the color density of the T line would be lighter.

Cr^{3+} standard solutions at various concentrations ranging from 0 ng/mL-80 ng/mL were prepared to determine the sensitivity of the assay. The water samples without addition of standard Cr^{3+} served as the control. As shown in Fig.5, the T line disappeared when the concentration of Cr^{3+} was more than 50ng/mL, indicating the visual LOD of ICA for Cr^{3+} was 50ng/mL.

The strips were also read by lateral flow reader. Fig. 6A shows the reflection light signals of the sensor with increasing Cr^{3+} concentrations. As shown, the lowest peak intensity changes with the increase of Cr^{3+} concentrations. The results demonstrated a good calibration curve was obtained in a wide range (Fig. 6B). A detection limit of 4.8 ng/mL was calculated as $90(F/F_0)$ (where F and F_0 are the lowest peak reflection light intensity obtained with the Cr^{3+} analyte and the blank sample, respectively, $Y = -0.011X + 0.9517$, $R^2 = 0.9920$). The reproducibility of this method was studied by four duplicated measurements of Cr^{3+} at different concentrations. Under current conditions, the relative standard deviations for F/F_0 from 5 to 80 ng/mL Cr^{3+} are 3.6%, 4.2%, 7.7%, 1.8%, 8.8%, and 6.7% respectively.

Compared with various methods reported for the determination of Cr, the present method offers the potential advantages of high sensitivity, simple operation and short testing time for Cr determination (Table 1).

Specificity and stability of the test strip

The cross-reactivity of the test strip with other metal ions (i.e. Fe^{3+} , Pb^{2+} , Hg^{2+} , Cu^{2+} , Al^{3+} , Mg^{2+} , Ca^{2+} , Zn^{2+} and Co^{2+}) was tested. Concentrations of the different metal ions from 0 to 1000 ppb were prepared for the assessment. The results indicated that the test strip has no cross-reactivity with these examined metal ions except a slight reactivity with Fe^{3+} .

The conformational changes of the chelate arms as they shift to accommodate various metal ions appear to be an important factor in antibody recognition. For example, the mAbs for Cd^{2+} usually have a cross-reactivity with Hg^{2+} because there is only a difference of 0.7 Å in the ionic radius between Cd^{2+} and Hg^{2+} . Moreover, the distance differences between atom positions in the Cd^{2+} -EDTA and Hg^{2+} -EDTA structures all fall below 0.2 Å, indicating that the three-dimensional structures of these two complexes are very similar.[34]

The ionic radius of Cr^{3+} , Fe^{3+} , and Al^{3+} are 0.615, 0.645 and 0.535 Å, there is 0.03 Å slight difference between Cr^{3+} and Fe^{3+} . Therefore, it's assumed that the ionic radius, the stereoisomeric conformation and the difference distance matrixes of Cr^{3+} -EDTA and Fe^{3+} -EDTA cause the slight cross reactivity.

The test strips obtained from the same batch were stored at different temperatures for various times to test the stability. According to Arrhenius equation, when the test strips were stored for 91 days at 37°C, the amount of samples were equal to that in the strips stored for 343.7 days at 25.1°C without significant lose of activity (Fig. 7).

Analysis of Cr^{3+} and Cr^{6+} in water samples

Different water samples from lakes and rivers in Guangzhou (Guangdong Province, China) were spiked with 0, 20, 50, 100 ng/mL of Cr^{3+} , and analyzed by the test strips. The samples were also spiked with 0, 20, 50, 100 ng/mL of Cr^{6+} , followed with the pretreatment for the reduction of Cr^{6+} to Cr^{3+} . As shown in Table 2, the results were consistent with those obtained by ICP-AES, suggesting the test strip could be used to analyze water samples of different matrices. Therefore, the test strip could be used as a portable method for the rapid detection of total chromium concentrations in water samples.

Analysis of Cr^{3+} in human serum samples

As mentioned above the serum Cr levels normally range from less than 50 up to 500 ng/mL. For serum testing, the sensitivity of the test strips were adjusted to 500 ng/mL through changing the amount of the gold-labeled anti-Cr-EDTA McAbs and the Cr-EDTA-BSA. Different pretreated human serum samples were analyzed by these test strips. The results show that the initial Cr^{3+} concentrations of the serum samples were below 500 ng/mL. Then the samples were spiked with 0, 200, 300, 400 ng/mL of Cr^{3+} and the test results were shown in Fig. 8. All serum specimens were measured by ICP-AES. As shown in Table 3, the results of two methods were consistent. It suggests that the test strip could be used to monitor Cr^{3+} levels in serum.

Conclusion

In this study, an immunochromatographic assay using gold nanoparticles coated with monoclonal antibodies for the detection of Cr^{3+} and Cr^{6+} in water and serum samples was successfully developed. It was the first time that test strip based assay was applied for the detection of Cr^{3+} and Cr^{6+} . The visual detection limit was as low as 50 ng/mL and the detection limit could reach 5 ng/mL when integrated with strip reader, which suggested that this method was sensitive enough for detecting of Cr ion in water. Moreover, the test strip was preliminarily investigated for Cr^{3+} detection in serum samples. Furthermore, test strip

based assay can be performed within 5 min. In summary, this study demonstrated that this method is rapid, sensitive, cost-effective and convenient for the on-site detection of chromium ions in environmental water samples and serum samples. In addition, heavy metals were reported to be in excess in other samples, such as soils, vegetables and tobacco, and this test strip could be used to analyze of these samples in the future.

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Fig 1.
Chemical structures of isothiocyanoethyl-EDTA, EDTA-Cr, EDTA-Cr-BSA.

Fig 2.
 (A) A schematic representation of the immunochromatographic assay. (B) The principle of negative detection. (C) The principle of positive detection.

Fig 3.
The sizes of naked gold nanoparticles were observed by TEM. The gold nanoparticles were scanned under a TEM ($\times 93000$) to measure the size.

Fig 4. UV-Vis spectra of naked colloidal gold nanoparticles and colloidal gold-labeled McAb. The red shift of maximum absorption wavelength demonstrated that anti-Cr-EDTA McAbs had been labeled successfully.

Fig 5. Sensitivity test of ICA for water samples. A series of concentrations of Cr^{3+} standard ranging from 0 ng/mL-80 ng/mL were prepared. Cr^{3+} at 0, 10, 30, 50 and 80 ng/mL was used as standard (Lane 1-5).

Fig 6.

(A). The reflection light signals of the sensor with increasing Cr^{3+} concentrations. From the bottom: 0, 5, 10, 30, 40, 50, 80, 100 and 120 ng/mL, respectively. (B). Standard inhibition curve of the reflection light signals. F and F_0 are the lowest peak reflection light intensity obtained with the Cr^{3+} analyte and the blank sample, respectively.

Fig 7.
The intensity of the test and control lines of test strips stored for 0, 28, 56, 77 and 91 days at 37°C.

Fig 8. Sensitivity test of ICA for serum samples. The initial concentration of Cr^{3+} in sample 5 is 119.1. Then the sample were spiked with 0, 200, 300, 400 ng/mL of Cr^{3+} . Cr^{3+} at 119.1, 319.1, 419.1 and 519.1 ng/mL was used as standard (Lane 5-1).

Table 1

Comparison of various methods for chromium ions detection

Technique	Linear range	Detection limit	Type of analyzed samples	Reference
HPLC	50 – 1000µg/L	3.4µg/L	Wastewater and Natural water	[25]
ETAAS	5–50µg/L	2.5µg/L	blood fractions samples	[26]
Fluorescence quenching	10–1000µg/L	8µg/L	water	[27]
CPE-GFAAS	0.02-20µg/L	0.02 µg/L	human serum	[28]
Fe ₃ O ₄ @ZrO ₂ nanoparticles-FAAS	4-400µg/L	0.69 µg/L	Environmental and biological samples	[29]
CPE-HPLC	50–5000µg/L	7.5µg/L	Environmental sediment samples	[30]
FI-FAAS	1–40µg/L	0.8µg/L	Natural water	[31]
AAS	—	1.1	water	[32]
SPME-GC-FPD	5–300 µg/L	2 µg/L	water	[31]
KinExA immunoassay instrument	—	1.6 µg/L	Water and serum	[33]
ICA	10-80 µg/L	4.8 µg/L	Water and serum	This work

Table 2

Comparison of immunochromatography strips with ICP-AES for analysis of environmental water samples spiked with Cr^{6+} and Cr^{3+}

	Fortified Chromium ions Concentration(ng/ mL)		ICP-AES (ng/ mL)		ICA	
	Cr^{6+}	Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}	Cr^{3+}
Water sample 1	0	0	6.0	18.2	-	±
	20	20	26.8	39.2	±	±
	50	50	56.4	68.5	+	+
	100	100	107.2	118.7	+	+
Water sample 2	0	0	8.2	23.8	-	±
	20	20	28.6	45.5	±	±
	50	50	57.9	71.6	+	+
	100	100	108.4	105.8	+	+
Water sample 3	0	0	5.4	16.7	-	±
	20	20	25.6	37.8	±	±
	50	50	56.5	65.7	+	+
	100	100	106.2	115.9	+	+
Water sample 4	0	0	3.5	14.8	-	±
	20	20	24.2	36.8	±	±
	50	50	52.8	65.2	+	+
	100	100	103.7	114.2	+	+

Water samples with four concentrations of fortified Cr^{6+} and Cr^{3+} were analyzed with the immunochromatography strips and with ICP-AES. (+) "Positive", Cr^{6+} and Cr^{3+} concentration was higher than 50 ng/ mL; (±) "weakly positive", Cr^{6+} and Cr^{3+} concentration was between 30 and 50 ng/ mL; (-) "negative", Cr^{6+} and Cr^{3+} concentration was below 20 ng/ mL.

Table 3

Comparison of immunochromatography strips with ICP-AES for analysis of human serum samples for Cr^{3+}

	ICP-AES (ng/ mL)	ICA	Fortified Chromium ions Concentration(ng/ mL)	ICA
Serum sample 1	233.7	–	300	+
Serum sample 2	163.5	–	300	±
Serum sample 3	116.6	–	300	±
Serum sample 4	228.6	–	300	+
Serum sample 5	119.1	–	300	±

Serum samples and serum samples with 300ng/mL of fortified Cr^{3+} were analyzed with the immunochromatography strips and with ICP-AES. (+) “Positive”, Cr^{3+} concentration was higher than 500 ng/ mL; (±) “weakly positive”, Cr^{3+} concentration was between 250 and 500 ng/ mL; (–) “negative”, Cr^{6+} and Cr^{3+} concentration was below 250 ng/ mL.