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Development and validation of an immunochromatographic assay for rapid detection of sulfadiazine in eggs and chickens

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Abstract

A rapid immunochromatographic assay (ICA) was developed and validated for the detection of sulfadiazine in eggs and chickens. Based on the competitive reaction mechanism, the competitor of sulfadiazine (sulfadiazine–BSA conjugate) was immobilized to the defined detection zone on a nitrocellulose membrane which acted as the capture reagent, and the monoclonal antibody against sulfadiazine was conjugated to colloidal gold particles which served as the detection reagent for the preparation of the immunochromatographic strips to test sulfadiazine. With this method, the semi-quantitative detection of sulfadiazine was accomplished in less than 15 min, with high sensitivity to sulfadiazine (5 ng/g) and low cross-reactivities with other sulfonamides. With experimental egg and chicken samples spiked with sulfadiazine at concentrations of 10, 20, and 100 ng/g, recoveries were demonstrated to be from 71% to 97% in egg samples and 71% to 95% in chicken samples. This method was compared with the enzyme-linked immunosorbent assay by testing 52 egg samples from the animal experiment, and compared with the high-performance liquid chromatographic method by testing 56 chicken samples, with an agreement rate of 100% for both comparisons, by using the maximum allowed residue of sulfadiazine (i.e. 100 ng/g) as the cut-off level as set by the European Union and China. The accuracy of ICA was also confirmed in an initial study with marketed egg and chicken samples. In conclusion, the method is rapid and accurate for the detection of sulfadiazine in eggs and chickens.

Keywords: Immunochromatographic assay; Colloidal gold; Strip; Sulfadiazine; Egg; Chicken

1. Introduction

Sulfonamides are often used for the prevention and control of a number of veterinary diseases. They are administered orally or mixed with animal feeds. As a result, sulfonamides can be present in food products of animal origin. Sulfonamide residues in food and animal tissues may be present in minute concentrations but may pose a health threat to consumers [1]. Sulfadiazine is an important ingredient in the sulfonamide residue products. To prevent potential health problems for consumers, the maximum allowed residue level (MRL) of sulfonamides has been established. For instance, in Europe and China, the MRL for the total amount of sulfonamides in edible tissues is set to be 100 ng/g [2,3]. Therefore, it is important to establish a validated method for the detection of such residues in meat and other animal by-products (milk and eggs) used for human consumption.

Several analytical methods have been developed for the analysis of sulfonamides, such as high-performance liquid chromatography (HPLC) [4,5], Gas chromatography–mass spectrometry (GC–MS) [6] and enzyme-linked immunosorbent assay (ELISA) [7]. HPLC and GC–MS are sensitive and specific, but are very laborious and expensive. They are suitable for confirming but not for screening a large number of samples.

Abbreviations: ICA, immunochromatographic assay; MRL, maximum residue level; BSA, bovine serum albumin; EU, European Union; HPLC, high-performance liquid chromatography; GC–MS, gas chromatography–mass spectrometry; ELISA, enzyme-linked immunosorbent assay; TEM, transmission electron microscopy; McAbSD4, monoclonal antibody against sulfadiazine; CGC, colloidal gold conjugate; G/Peak-ROD, G/Peak-relative optical density; S.D., standard deviation; RC, recovery; CV, coefficient of variation

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Therefore, a rapid, sensitive, specific and inexpensive assay is needed to detect positive samples in routine screening, which can be further confirmed for the presence of sulfonamides by other methods such as HPLC. A variety of immunoassays have been developed recently [8–10]. ELISA is an efficient immunoassay, which can be used for the analysis of a large volume of samples, but it requires fussy operations including incubation, washing and enzymatic reactions during signal generation. More recently, a novel method called immunochromatographic assay (ICA) has increasingly been used in the detection of several chemicals, which is a one-step immunoassay and does not require fussy operations as in ELISA [11–14]. We first reported herein the utilization of an anti-sulfadiazine monoclonal antibody conjugated with colloidal gold to develop an immunochromatography strip for the semi-quantitative detection of sulfadiazine in eggs and chickens. As confirmed by the results from ELISA and HPLC, ICA assay is shown to be rapid, simple, and effective for the rapid detection of sulfadiazine.

2. Experimental

2.1. Equipment

The transmission electron microscopy (TEM) images were recorded with a Hitachi H600 transmission electron microscope (Hitachi Instrument Co., Tokyo, Japan). UV–visible spectra were obtained by using an 8453 UV/Visible Spectrophotometer (Aligent Technologies, Palo Alto, USA). ZX1000 Dispensing Platform and CM4000 Guillotine Cutter (BioDot, Irvine, USA) were used to prepare test strips. TSR3000 Test Strip Reader (BioDot) was used to analyze the intensity of test strips. The samples were evaluated by 2695 Alliance HPLC System (Waters Co., Milford, USA). ELX800 Universal Microplate Reader (BIO-TEK Instrument INC., Winooski, USA) was used to measure the optical density of the microplate.

2.2. Chemicals and reagents

Gold chloride ($HAuCl_4 \cdot 3H_2O$), sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), bovine serum albumin (BSA), polyvinylpyrrolidone K30 and sulfadiazine were purchased from Sigma (St. Louis, MO, USA). The goat anti-mouse antibody was obtained from Sino-American Biotechnology Co. (Luoyang, China). Sulfadiazine–BSA conjugate and the monoclonal antibody against sulfadiazine (McAbSD4) were prepared in our laboratory [15]. Nitrocellulose membranes, glass fibers and absorbent paper were purchased from Millipore Corporation (Bedford, MA, USA). The enzyme-linked immunosorbent assay (ELISA) kit for sulfadiazine analysis was obtained from RANDOX Laboratories Ltd. (Ardmore, Diamond Road, Crumlin, Co., Antrim, United Kingdom).

2.3. Sample materials

2.3.1. Experimental egg samples

The hens were randomly divided into control and test groups. The control group ($n = 2$) was not treated with sulfadiazine and the test group ($n = 5$) was treated with feeds containing sulfadiazine (400 mg/kg) for five consecutive days, which were then maintained without sulfadiazine treatment for 8 days. During the 8-day period, all the eggs from the control and test group were collected daily, and the eggs collected from the control group were used as control samples. All egg samples were subject to ICA and ELISA for sulfadiazine analysis.

2.3.2. Experimental chicken samples

The broilers were also randomly divided into control and test groups. The control group ($n = 16$) was not treated with sulfadiazine and the test group ($n = 40$) was treated with feeds containing sulfadiazine (500 mg/kg) for 7 days, which were then maintained without sulfadiazine treatment for 3 days. Five broilers from the test group and two broilers from the control group were slaughtered at days 1, 3, 5, and 7 during the treatment period and 12, 24, 48 and 72 h after the withdrawal of the sulfadiazine feeds. The muscle samples (100 g per sample) were collected and used for sulfadiazine analyses by ICA and HPLC.

2.3.3. Samples from marketed eggs and chickens

One hundred and forty egg samples and 100 chicken samples were randomly collected from markets in some city, China, in March 2006 and tested by the ICA method developed in this study.

2.3.4. Sample pretreatment

The sample pretreatment was accomplished by sample homogenization, extraction, and drying. Each chicken or egg sample was homogenized in ethyl acetate (2 ml/g wet weight). The homogenate was vortexed for 3 min and then centrifuged at $2000 \times g$ for 10 min. The resultant supernatant (300 μl) was evaporated to dryness by heating at 60 °C or in a 60 °C water bath under a gentle flow of nitrogen. The residue was re-suspended in 150 μl of phosphate buffer (pH 7.4) and mixed thoroughly before analysis.

2.4. Synthesis of colloidal gold

Colloidal gold was prepared by using a previously reported method [16] with slight modifications. In a 500-ml round-bottom flask, 200 ml of 0.01% (w/w) $HAuCl_4$ in doubly distilled water was brought to a boil with vigorous stirring, and 4 ml of 1% trisodium citrate was then added to the solution. The solution turned deep blue within 20 s and the color changed to wine-red 60 s later. After continued boiling for an additional 10 min, the heating source was removed and the colloid was stirred for 15 min. The colloidal gold solution was stored at 4 °C in a dark-colored glass bottle until use.

2.5. Preparation of colloidal gold–McAb conjugate

McAbSD4 was purified from mouse ascitic fluid by using caprylic acid and ammonium sulfate [17,18] dialyzed against the 2-mM phosphate buffer (pH 7.4) at 4 °C for two days, and then conjugated with a colloidal gold to generate McAbSD4-CGC (colloidal gold conjugate). Briefly, 30 mg of the purified

McAbSD4 in 0.5 ml distilled water was added to 10 ml of gold colloid solution (adjusted to pH 8.0), and the mixture was stirred vigorously for 30 min, to which 2.5 ml of 5% (w/v) BSA aqueous solution was added to block excess reactivity of the gold colloid, followed by stirring for 30 min. After centrifugation at 12,000 × *g* at 4 °C for 30 min, the supernatant was removed by aspiration, and the resultant McAbSD4-CGC pellet was then suspended in 2-mM borax buffer (pH 9.0) containing 0.1% (w/v) PEG-20000 and washed twice with the same buffer, before the final pellet was re-suspended in 1 ml of the same buffer.

2.6. Preparation of the immunochromatography strip

A schematic diagram for the preparation of the sulfadiazine immunochromatography strip is shown in Fig. 1 The sample (absorbent paper) and the conjugate pads (glass–fiber membrane) were treated with 20 mM phosphate buffer containing 2% BSA, 2.5% sucrose, 0.3% polyvinylpyrrolidone K30 and 0.02% sodium azide, pH 7.4, and dried at 37 °C. Subsequently, sulfadiazine–BSA (0.125 mg/ml) and the goat anti-mouse antibody (1 mg/ml) were applied to the nitrocellulose membrane

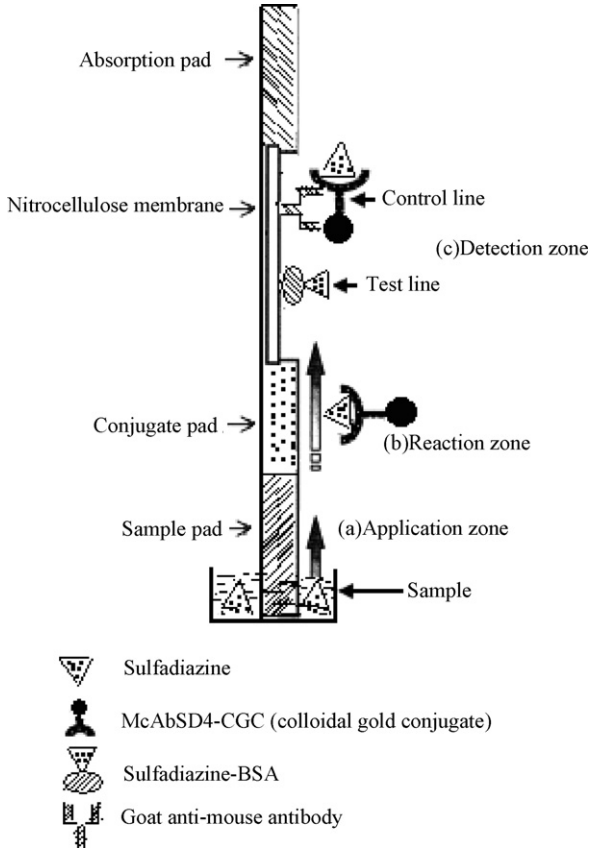

Fig. 1. The schematic diagram for the preparation of the immunochromatographic strip for sulfadiazine. (a) Application zone; (b) reaction zone; (c) detection zone. The strip consists of a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorption pad. The conjugate pad contains McAbSD4-CGC (colloidal gold conjugate). In the detection zone, the nitrocellulose membrane is used as a chromatographic support on which the sulfadiazine–BSA and goat anti-mouse antibody are immobilized.

to the test and control lines by using the BioDot XYZ Platform at a jetting rate of 0.75 μl/cm and then dried at 37 °C. The absorption pad (absorbent paper) was used without treatment. The McAbSD4-CGC was applied to the treated conjugate pad at a jetting rate of 6 μl/cm and completely lyophilized. The absorption pad, nitrocellulose membrane, pretreated conjugate pad, and the sample pad were assembled as the strip and attached to a plastic scaleboard. Then, they were cut into 65-mm-long and 3-mm-wide strips. The application zone of strips were put in a 120-μl aliquot of sulfadiazine samples for analysis.

2.7. Principle of immunochromatographic assay and quantification of sulfadiazine

The assay is based on the competitive reaction theory. When a sample is applied to the sample pad, it rapidly wets through to the conjugate pad, and the detector reagent (MAbSD4-CGC) is then solubilized. The detector reagent begins to migrate along with the sample flow front up the nitrocellulose membrane. In the absence of sulfadiazine in the test sample, when the sample passes over the test line to which sulfadiazine–BSA is immobilized, the detector reagent (McAbSD4-CGC) is bound and the excess detector reagent is trapped by the control line. Two red bands at the test and control lines are then developed. In contrast, when the sample contains sulfadiazine, it will bind to the detector reagent, and, no band or one baby-red band which is weaker than the band of sulfadiazine of the negative control sample at the test line is present. The result can be visualized by naked eyes, and the intensity of the test line is in proportion to the amount of sulfadiazine present in the samples. Several concentrations of sulfadiazine (5, 10, 20, 40, 80, and 100 ng/ml) and the negative control were included in the preparation of standard curves. The intensity of their test lines was obtained by using the test strip reader and the quantitative values were expressed as G/Peak-relative optical density (G/Peak-ROD). The standard curves were constructed by plotting the G/Peak-ROD values obtained from each reference standard against corresponding concentrations in ng/ml. The G/Peak-ROD value was used to determine the concentration of the tested samples from the standard curve. The total assay time was less than 15 min.

2.8. Statistical analysis

Data were presented as mean values ± standard deviation (S.D.). Student's *t*-test was used for the determining the difference between the groups. SPSS software 12.5 for windows (SPSS Inc., Chicago, IL, USA) was used for data analysis. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characterization of the colloidal gold particles

The diameter of colloidal gold was obtained by UV–vis measurements and examination under TEM. The UV–vis spectroscopic analysis yielded a maximum absorbance at 520 nm (Fig. 2) and the TEM images of the gold particles showed

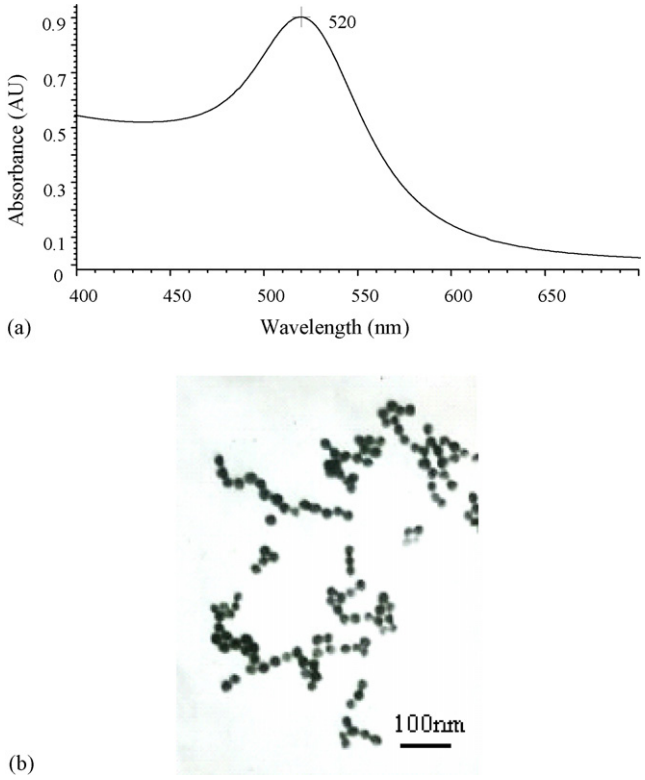

Fig. 2. UV–visible spectra of colloidal gold solution (a) and TEM image of colloidal gold particles (b). The maximum absorbance of the colloidal gold solution is 520 nm and the size of the colloidal gold particles is 18.6 ± 1.2 nm (100 particles sampled).

the average diameters of the colloidal gold particles to be 18.6 ± 1.2 nm ($n = 100$). These results indicate that the preparation of colloidal gold particles meet the requirements for the preparation of colloidal gold conjugate and signal generation in ICA.

3.2. Monoclonal antibody

Using the ELISA procedure as described previously with the monoclonal antibody against sulfadiazine (McAbSD4), a dose–response curve was obtained for reference sulfadiazine standards ranging from 0 to 1000 ng/ml (Fig. 3). The 50% inhibition of sulfadiazine was obtained at 25.6 ng/ml. The 50% inhibitions of major sulfonamides were also measured. Then the cross-reactivities of the antibody with a range of sulfonamides were established (Table 1). The low cross-reactivities were found for sulfamonomethoxine (1.3%), sulfametoxydiazine (1.0%), sulfadimethoxine (0.6%), sulfamethazine (<0.1%), sulfaquinoxaline (<0.01%), and sulfamethoxazole (<0.01%). Therefore, McAbSD4 is suitable for use in ICA to detect sulfadiazine because of its low cross-reactivities with major sulfonamides.

3.3. Test sensitivity

Each standard sample containing various concentrations of sulfadiazine (5, 10, 20, 40, 80 and 100 ng/ml) and each negative control sample were assayed eight times by using the

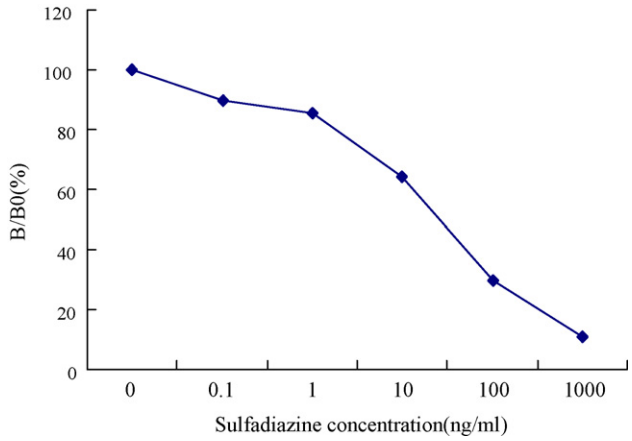

Fig. 3. The standard curve of the monocolonal antibody against sulfadiazine by competitive direct ELISA. Mean optical density values ($n = 10$) of each reference standard were measured by a microplate reader. The inhibition (B/B_0) = optical density of reference standard/optical density of zero standard. A standard curve can be constructed by plotting the inhibition obtained from each reference standard against its concentration in ng/ml on a standard curve. The 50% inhibition of sulfadiazine was 25.6 ng/ml.

methods as described previously. The color intensity of the test line was confirmed by direct visualization and quantified by the test strip reader. The results were expressed as G/Peak-relative optical density (G/Peak-ROD). In the analysis, the samples containing 5 ng/ml or more of sulfadiazine displayed positive signals ($P < 0.01$). The linearity was seen between 0 and 40 ng/ml ($R^2 = 0.97$) (Fig. 4). The test band could be visualized with the naked eyes. Based on the color and its density, semi-quantitative results can be determined. If the test band with red color was similar to the color of the negative control present at the test line and thus the sample would be considered to be negative (−) when sulfadiazine concentration was less than 10 ng/g. The test band with baby-red color was weaker than the red band of the negative control when more sulfonamide presented in the sample, and thus the sample would be considered to be weakly positive (±) when sulfadiazine concentration was in the range of 10–100 ng/ml. When there was no band at the test line, the sample was positive (+) when sulfadiazine concentration was greater than 100 ng/ml. These results indicate that our test strip detects sulfadiazine with a sensitivity of at least 5 ng/g for samples, if determined by the strip reader. If determined visually, the sensitivity was considered to be 10 ng/g for samples.

Table 1
Cross-reactivity data for the monoclonal antibody against sulfadiazine (McAbSD4)

Compounds	Cross-reactivity (%)
Sulfadiazine	100
Sulfamonomethoxine	1.3
Sulfametoxydiazine	1.0
Sulfadimethoxine	0.6
Sulfamethazine	<0.1
Sulfaquinoxaline	<0.01
Sulfamethoxazole	<0.01

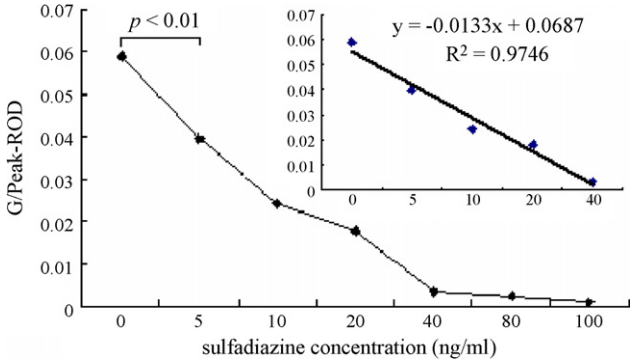

Fig. 4. Test sensitisity. Negative control samples and five standard samples (5, 10, 20, 40, 80 and 100 ng/ml) were assayed in test strips eight times each. Test line color intensity in strip was expressed as G/Peak-relative optical density (G/Peak-ROD). Data are presented as the mean ± S.D. of eight measurements. The coefficient variations (CV) of test line color intensity were 8.98%, 6.55%, 9.88%, 6.11%, 8.57%, 12.5% for negative control samples and standard samples of 5, 10, 20, 40, 80 and 100 ng/ml, respectively. The linearity was good from 0 to 40 ng/ml which has a linear regression equation ($y = -0.0133x + 0.0687$) and acceptable correlation ($R^2 = 0.97$).

3.4. Assay precision

Four standard samples with sulfadiazine concentrations in the range of 5–40 ng/ml were assayed to evaluate the precision of our assay. For the intra-day precision study, 10 repeated analyses were conducted with each sample in the same day. Similarly, 10 repeated analyses were performed with each sample daily in seven successive days for the inter-day precision study. The coefficients of intra-day variations (CV) were 6.1–9.9%, and the inter-day CVs were 6.9–10.3% (Table 2).

3.5. Recovery of sulfadiazine in egg and chicken samples

Non-infected egg and chicken samples which were confirmed by HPLC were spiked with sulfadiazine with concentrations at 10, 20, and 100 ng/g. Each spiked sample was assayed 8 times by using the test strip. The color intensity of the test band was analyzed by the test strip reader, and the values of samples were obtained using the standard curve as above. Recoveries were determined for each spiked sample and the mean recoveries (RC% ± S.D.) between 71% and 97% (CV%; between 2.9% and 12.8%) are shown in Table 3. As determined visually, one band with red color which was weaker than the red band of the negative control sample present at the test line for spiked samples with sulfadiazine at concentrations of 10 and 20 ng/g, and there was no band at the test line for those of 100 ng/g.

Table 2
Precision of the assay

Sample (ng/ml)	CV (%)	
	Intra-day	Inter-day
5	6.6	7.2
10	9.9	10.3
20	6.1	6.9
40	8.6	9.1

CV, coefficient of variation.

Table 3
Recovery (RC% ± S.D.) of sulfadiazine in egg and chicken samples

	RC% ± S.D. (CV%)		
	10 ng/g	20 ng/g	100 ng/g
Egg	71 ± 3 (4.2)	79 ± 9 (11)	97 ± 4 (4.3)
Chicken	71 ± 5 (7.4)	73 ± 9 (12.8)	95 ± 3 (2.9)

RC% ± S.D., recovery percentage ± standard deviation ($n = 8$). CV, coefficient of variation.

3.6. Comparison of ICA with ELISA and HPLC

3.6.1. Comparison of ICA with ELISA

A side-by-side comparison between ICA and ELISA was accomplished with 52 egg samples from the animal experiment. Two cut-off levels for the content of sulfadiazine in the samples were set at 10 and 100 ng/g for semi-quantitative detection. By visualization as described above, samples were determined to be negative (−, less than 10 ng/g), weakly positive (±, 10–100 ng/g), or positive (+, greater than 100 ng/g), respectively (Table 4). For ICA methods, 19 samples were negative (−); 8 samples were weakly positive (±); and 25 samples were positive (+). For ELISA methods, 16 samples were negative (−); 11 samples were weakly positive (±); and 25 samples were positive (+). Based on the maximum allowed residue level of sulfadiazine (100 ng/g) established by the European Union (EU) and China, the agreement rate between ICA and ELISA was 100%. Thus, we conclude that the ICA results are comparable with those of ELISA.

3.6.2. Comparison of ICA with HPLC

Similarly, a side-by-side comparison between ICA and HPLC was accomplished with 56 chicken samples from the animal experiment, with 10 and 100 ng/g as the cut-off points for semi-quantitative detection (Table 5). For ICA methods, 25 samples were negative (−); 7 samples were weakly positive (±); and 24 samples were positive (+). For HPLC methods, 29 samples were negative (−); 3 samples were weakly positive (±); and 24 samples were positive (+). Based on the maximum allowed

Table 4
Sulfadiazine detection using ICA and ELISA kit

ICA	ELISA			
	Positive (+)	Weakly positive (±)	Negative (−)	Total
Positive (+)	25	0	0	25
Weakly positive (±)	0	8	0	8
Negative (−)	0	3	16	19
Total	25	11	16	52

Two cut-off points was set at 10 and 100 ng/g. Negative (−) showed the concentration of sulfadiazine was less than 10 ng/g, weakly positive (±) showed that was 10–100 ng/g, and positive (+) showed that was more than 100 ng/g.

Table 5
Sulfadiazine detection using ICA and HPLC

ICA	HPLC			
	Positive (+)	Weakly positive (±)	Negative (−)	Total
Positive (+)	24	0	0	24
Weakly positive (±)	0	3	4	7
Negative (−)	0	0	25	25
Total	24	3	29	56

Two cut-off points was set at 10 and 100 ng/g. Negative (−) showed the concentration of sulfadiazine was less than 10 ng/g, weakly positive (±) showed that was 10–100 ng/g, and positive (+) showed that was more than 100 ng/g.

residue level of sulfadiazine (100 ng/g) established by the European Union (EU) and China, the agreement rate between ICA and HPLC was 100%. Thus, we conclude that the ICA results are closely aligned with those of HPLC.

3.7. Application of ICA in clinical testing

Among the 140 egg and 100 chicken samples collected from markets, 3 egg samples and 1 chicken sample were shown to be positive (+) for sulfadiazine, and 1 chicken sample was shown to be weakly positive (±) for sulfadiazine as tested with the ICA method. The concentrations of sulfadiazine were 229, 112, and 125 ng/g, respectively, in the three egg samples and 138 and 56 ng/g in the chicken sample, as measured by HPLC.

4. Discussion

To prevent potential health problems for consumers and to follow relevant laws and regulations, sulfonamides in food products of animal origin are routinely monitored. HPLC and ELISA, which are most commonly used to detect sulfadiazine in edible tissues, require expensive instruments and are labor- and time-consuming. On the other hand, immunochromatographic assay (ICA) has several advantages. There is no need for complex operations and therefore the detection time is remarkably shortened. Furthermore, semi-quantitative detection can be realized by the intensity of signals as a response to an analyte concentration. In particular, the results can be read directly by naked eyes, ensuring the convenience of assay on-site. Therefore, ICA can accelerate the analytical procedure and also provide a means for performing the test without reagent handling, allowing a one-step assay [19]. Here we developed an ICA to perform semi-quantitative analysis of sulfadiazine residue.

In order to develop sensitive and rapid detection methods for sulfadiazine, we adopted the competitive reaction format. Sulfadiazine–BSA conjugate was immobilized to a defined detection zone on the porous nitrocellulose membrane, and McAbSD4-CGC (colloidal gold conjugate) served as a detection reagent for the preparation of an immunochromatographic strip. In the competitive reaction, the proportion of sulfadiazine–BSA is an important factor to improve the sensitivity which could determine the competitive ability of sulfadiazine–BSA and sulfadiazine with MAbSD4-CGC and then affect the sensitivity of the assay. From several different proportions (20:1, 10:1, and 5:1), we selected a suitable proportion (10:1) to improve the sensitivity to 5 ng/g.

Although ICA is designed for semi-quantitative analysis, it requires a reading device [12]. In order to overcome this problem, we developed a novel method to differentiate the results (positive, weakly positive and negative) by identifying the color and its density in different test zones on the test strip. When the concentrations of sulfadiazine are in the range of 5–10 ng/g, the results are obtained by using a test strip reader. When the concentrations of sulfadiazine are in the range of 10–100 ng/g, the baby-red color, which is much weaker than the band of negative control sample, is present at the test zone, can be easily visualized by naked eyes. More importantly, when the concentrations of sulfadiazine are greater than 100 ng/g, there is no band at the test zone, which can be easily implemented to follow the regulation on the maximum allowed residue level of sulfadiazine (100 ng/g) as established by the European Union (EU) and China.

Reliable sample preparation is essential to prevent false negativities. However, a sample clean up is very laborious for accurate quantitative analysis by HPLC and GC–MS. Here we used a simple method to extract sulfadiazine from egg and chicken samples for ICA. Sulfadiazine was extracted with ethyl acetate, and the extracts were dried by heating in at 60 °C or in a 60 °C water bath under a gentle flow of nitrogen. The residue was suspended with phosphate buffer (pH 7.4). Using this method, we had good recoveries ranging from 71% to 97% in egg samples and from 71% to 95% in chicken samples. Meanwhile, our study indicated good recoveries from milk (92%), honey (98%), porcine muscle (96%) and liver (94%) at the concentration of 100 ng/g (data not shown), but the results are yet to be confirmed by animal experiments.

In practice, our ICA could perform quantitative detection of sulfadiazine based on the standard curve. But in routine screening, it would be more convenient and efficient to perform the semi-quantitative detection. In the comparison of test strip results with ELISA and HPLC results, we set up 10 and 100 ng/g as the cut-off points to differentiate the results (positive, weakly positive and negative) for semi-quantitative detection. In the comparison, three egg samples which were weakly positive (±) for ELISA were negative (−) for ICA, but the sulfadiazine concentrations were only 12, 13, and 13 ng/g for ELISA, respectively; four chicken samples which were weakly positive (±) for ICA were negative (−) for HPLC, but the sulfadiazine concentrations were only 12, 12, 13, and 11 ng/g, based on the ICA standard curve, respectively. Based on the maximum allowed residue level of sulfadiazine (100 ng/g), the agreement rates were 100%. Thus, we conclude that the ICA results are closely aligned with those of ELISA and HPLC.

In conclusion, we developed a specific ICA for sulfadiazine semi-quantitative detection, which has low cross-activities with other common sulfonamides. It is expected that an ICA technique that is able to efficiently and accurately detect all sulfonamides can be developed in the future.

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