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Monoclonal antibody-based ELISA and colloidal gold-based immunochromatographic assay for streptomycin residue detection in milk and swine urine*

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Abstract: A protein conjugate of streptomycin (streptomycin-bovine serum albumin (BSA) conjugate) was prepared and used as immunogen to produce monoclonal antibodies (MAb). One hybridoma secreting anti-streptomycin MAb was obtained and then used to produce MAb. The MAb named 13H5 showed the 50% maximal inhibitory concentration (IC_{50}) value of 4.65 ng/ml and the IC_{20} value of 0.21 ng/ml in phosphate buffered saline (PBS). At optimum conditions, an indirect competitive enzyme-linked immunosorbent assay (ELISA) and a colloidal gold-based immunochromatographic assay (CGIA) were developed and applied to detect streptomycin residues in milk and swine urine samples. The developed ELISA showed that the minimum detection limit was 2.0 and 1.9 ng/ml for milk and swine urine samples, respectively, without obvious cross-reactivity to other tested antibiotics except dihydrostreptomycin which gave a 118.32% cross reaction value. Milk and swine urine samples spiked with streptomycin at 10, 50, 100 and 200 ng/ml were analyzed by the established ELISA. The mean recovery of streptomycin was from 81.9% to 105.5% and from 84.3% to 92.2% for milk and swine urine, respectively. The optimized CGIA showed that the minimum detection limit was 20.0 ng/ml for milk and swine urine samples. The results of spiked analysis and specific analysis demonstrate that the CGIA could be applicable for screening milk and swine urine samples for the presence of streptomycin residues on-site. The established ELISA and CGIA allow the rapid, low-cost, and sensitive determination of streptomycin residues in food samples.

Key words: Streptomycin, Monoclonal antibody, Antibiotic residue, Enzyme-linked immunosorbent assay (ELISA), Colloidal gold-based immunochromatographic assay (CGIA)

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1 Introduction

Antibiotics are particularly important feed additives in animal confinement housing to increase feed efficiency, promote growth, and prevent diseases from spreading among the confined animals. Streptomycin is an aminoglycoside antibiotic with well-known antituberculosis activity, and has been widely used in veterinary medicine for treatment of Gram-negative bacteria infections because of its effectiveness and low cost (Unusan, 2009). Streptomycin and its analogue aminoglycoside antibiotics act principally by impairing bacterial protein synthesis through binding to prokaryotic ribosomes (Mingeot-Leclercq *et al*., 1999). However, streptomycin has the potential for severe side-effects, such as streptomycin-caused ototoxicity, allergic reaction, and inhibiting growth of marrow. Furthermore, there is present concern about streptomycin residues in the environment and food due to the potential for the increased incidence of drug resistance among pathogenic micro-organisms (Ingerslev and Halling-Sørensen, 2001). The use of antibiotics in animal agriculture has been linked to the increased emergence of resistant strains of pathogenic

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bacteria that could potentially impact human health (Molbak *et al*., 1999; Smith *et al*., 1999; Pérez *et al*., 2007). For consumer protection, regulatory authorities have established residue limit for streptomycin in edible tissues and milk. In Europe, the European Commission, Regulation 2377/90 has established the maximum streptomycin residue limit (MRL): 200 mg/kg in milk, 500 mg/kg in meats, and 1000 mg/kg in kidney. It is thus necessary to monitor the concentration of the streptomycin residues in food samples.

At present, three methods for antibiotic residue detection are primarily applied: microbiological assay, instrument method, and immunoassay method. Microbiological assay is used for screening antibiotics in food because of its convenience, low costs, and broad spectrum characteristics (Haasnoot *et al*., 1999; 2002). But, this assay is currently done in a laboratory and is slow, with a low sensitivity (Verheijen *et al*., 2000). The current instrument methods used to analyze streptomycin are gas chromatography, high performance liquid chromatography (HPLC), and liquid chromatography with mass spectrometric detection (LC-MS) (Abbasi and Hellenas, 1998; Preu and Petz, 1999). These methods are sensitive and highly specific, but require expensive instrumentation and highly skilled analysts. They are time-consuming and expensive, and are not suitable for routine analysis of large-scale samples. Immunoassay has been an alternative to the instrument and microbiological methods for accurate measurements of antibiotic residues in complex matrices, because it is highly sensitive and specific, can be conducted on a large scale, is of low cost, and is rapid and simple to conduct. Unlike the instrument methods, immunoassays do not require sample pre-concentration and extraction (Abuknesha and Luk, 2005), so they can be used extensively in detecting trace amounts of chemicals such as antibiotics and pesticides (Aga *et al*., 2003; Abuknesha and Luk, 2005; Jin *et al*., 2008; Qian *et al*., 2009).

Immunoassays for measuring streptomycin residues in animal samples based on polyclonal antibodies and monoclonal antibodies (MAb) have been described (Schnappinger *et al*., 1993; Heering *et al*., 1998; Haasnoot *et al*., 1999; Verheijen *et al*., 2000; Watanabe *et al*., 2002; Abuknesha and Luk, 2005; Samsonova *et al*., 2005). In this paper, an indirect competitive enzyme-linked immunosorbent assay (ELISA) and colloidal gold-based immunochromatographic assay (CGIA) using an anti-streptomycin MAb were developed and applied to detect streptomycin and dihydrostreptomycin residues in milk and swine urine samples. It was again demonstrated that the immunoassay established is suitable for detecting (dihydro)streptomycin residues in animal samples.

2 Materials and methods

2.1 Reagents and animals

Bovine serum albumin (BSA), ovalbumin (OVA), streptomycin sulfate, dihydrostreptomycin sulfate, neomycin sulfate, gentamicin sulfate, kanamycin sulfate, penicillin G, sulfamethazine and chlortetracycline, pristane, Freund's complete and incomplete adjuvants, carboxymethoxylamine hemihydrochloride (CMO), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), gold chloride trihydrate, and horseradish peroxidase (HRP)-labelled goat anti-mouse IgG were purchased from Sigma-Aldrich (St. Louis, MO, USA). The murine myeloma cells Sp2/0 were grown in the basic medium of RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 20% (v/v) fetal calf serum. Eight-week-old specific-pathogen-free BALB/c mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China.

2.2 Equipment

Ninety-six-well flat ELISA plates and 96-well flat cell culture plates (catalogue No. 3599) were supplied by Corning Inc. (Cambridge, MA, UK). The model 680 microplate reader was supplied by Bio-Rad (HERCULES, CA, USA). A BioDot system dispensing platform (BioDot Inc., Irvine, CA, USA) and a model SS-4 automatic guillotine cutter (Elmwood Park, NJ, USA) were used to prepare the immunochromatographic strip.

2.3 Preparation of streptomycin with carrier protein conjugates

Streptomycin-BSA conjugate was used as the immunogen and the test line capture reagent was prepared as described previously by Schnappinger *et al*. (1993). Streptomycin-OVA conjugate was prepared by a similar procedure using OVA instead of BSA.

2.4 Immunization

Eight 8-week-old BALB/c female mice were vaccinated intraperitoneally with emulsion containing Freund's complete adjuvant and streptomycin-BSA conjugate (1:1, v/v; a dose of 150 μg streptomycin-BSA conjugate per mouse). Four similar booster vaccines were given at 3-week interval, but the immunogen was emulsified in Freund's incomplete adjuvant. Three days before cell fusion, the mouse was immunized intraperitoneally with 300 μg streptomycin-BSA conjugate without adjuvant.

2.5 Preparation of hybridomas

The cells were fused as described by Yu *et al*. (2005). The fused cells were cultured and selected in twenty 96-well cell culture plates with RPMI 1640 medium containing hypoxanthine-aminopterin-thymidine (HAT, Sigma-Aldrich) and 20% (v/v) fetal calf serum. At 10 d after cell fusion, the medium was replaced with RPMI 1640 medium containing hypoxanthine-thymidine (HT, Sigma-Aldrich) and 20% (v/v) fetal calf serum. At 13 d after cell fusion, anti-streptomycin antibodies in hybridoma culture supernatants were detected with indirect ELISA and indirect competitive ELISA using streptomycin-OVA as a coating antigen. Hybridomas secreting highly specific and sensitive anti-streptomycin antibodies were selected and cloned with the method of the limiting dilution for more than three times to get the hybridoma lines. Stable antibody-secreting clones were expanded and stored in liquid nitrogen.

2.6 Production of MAb

The hybridomas were injected intraperitoneally into pristane-primed BALB/c mice to prepare the ascites. After 7–10 d the ascites samples were collected and their titres were detected with indirect ELISA using streptomycin-OVA conjugate as a coating antigen (Wu *et al*., 2007). The class and subclass of the isotypes of the MAb were decided by double immunodiffusion assay with a mouse MAb isotyping kit according to the manufacturer's instruction (Sigma-Aldrich). Anti-streptomycin IgG was purified from ascites with an immobilized protein-G affinity column (GE Healthcare, Bucks, UK) according to the manufacturer's manual. Purified antibodies were stored at −80 °C.

2.7 Indirect competitive ELISA and indirect ELISA

Bidimensional titration assays were used to determine the most appropriate antibody concentration and the most suitable coating antigen concentration for the competitive ELISA. Indirect competitive ELISA was carried out following the standard procedures (Jin *et al*., 2008). Briefly, the wells of ELISA plate were coated with streptomycin-OVA conjugate at an appropriate concentration in sodium carbonate buffer (50 mmol/L, pH 9.6) and then coated at 4 °C for overnight. The coating wells were blocked with 3% dried skimmed milk in phosphate buffered saline (PBS, 300 μl/well) for 30 min. Fifty microliters of the competitor (standard streptomycin sulfate or tested antibiotics or samples) and 50 μl of MAb at the most suitable concentration were added while the plates were incubated for 1 h at 37 °C. The most appropriate dilution HRP-labelled goat anti-mouse IgG (100 μl/well) was added and incubated for 1 h at 37 °C. Each step was followed by four-time washing of the plates with PBS containing 0.05% (v/v) Tween-20. The HRP tetramethylbenzidine (TMB) substrate solution (100 μl/well) was added and reacted for 15 min at 37 °C. Two mol/L H_2SO_4 (50 μl/well) was used to terminate the enzyme reaction. The absorbance value at 450 nm was detected with a Bio-Rad model 680 microplate reader (Bio-Rad, Hercules, CA, USA). According to the absorbance and the logarithm of analyte concentration, the standard curves were established. Data were calculated using Microsoft® excel 2007. The 50% maximal inhibitory concentration (IC_{50}) and IC_{20} values were calculated based on sigmoidal curves, which were obtained based on a four-parameter logistic equation (Raab, 1983).

The indirect ELISA process was similar to the indirect competitive ELISA except that, in the indirect ELISA process, 100 μl of hybridoma culture supernatants or diluted antibodies after blocking were added to the ELISA plate wells, as described by Wu *et al*. (2007).

2.8 Cross-reactivity of MAb

Several structurally related or commonly used antibiotics (dihydrostreptomycin, neomycin, gentamycin, kanamycin, penicillin G, sulfamethazine and

chlortetracycline) were used for the MAb cross-reactivity analysis with the indirect competitive ELISA as described as the above. The equation of cross-reactivity (CR) was expressed as follows (Abad *et al*., 1999): *CR* (%)=(IC_{50} of streptomycin/IC_{50} of the tested antibiotics)×100.

2.9 Recovery of spiked samples

Milk and swine urine, for use as streptomycin-free samples, were purchased from a local farm and were identified for the absence of streptomycin with HPLC. The milk and swine urine samples were centrifuged at 5000 r/min for 5 min to remove the fat and precipitate. To make calibration curves of streptomycin in milk and swine urine, streptomycin stock standard solution (1000 μg/ml) was prepared by dissolving streptomycin sulfate in PBS. The stock standard solution was diluted with milk or swine urine to 0, 0.3, 1, 4, 10, 20, 50, 100, 200, 400, 800 and 1000 ng/ml, which were further diluted 5-fold in PBS (10 mmol/L, pH 7.4) in order to neglect the matrix effects on the immunoassays. The calibration curve of streptomycin in milk or swine urine was prepared with indirect competitive ELISA. For the recovery assay, streptomycin-spiked solutions were prepared by dissolving streptomycin sulfate in milk or swine urine to obtained final concentrations of 10, 50, 100 and 200 ng/ml, which were further diluted 5-fold with PBS. The recovery of streptomycin from the spiked milk or swine urine was obtained on the basis of the calibration curve prepared by the indirect competitive ELISA.

2.10 CGIA

2.10.1 Colloidal gold-labelled MAb

Forty-nm-size colloidal gold was prepared as described by Grabar *et al*. (1995). The colloidal gold-labelled MAb was prepared as described by Verheijen *et al*. (2000) and the optimum MAb concentration for labelling was determined as described by Zhang *et al*. (2006). Briefly, 800 μg of the purified MAb in 1 ml Milli-Q purified water was slowly added to 100 ml of colloidal gold solution (pH 8.0) and the mixture was stirred vigorously for 20 min at room temperature. Then 10 ml of 5% (w/v) BSA solution was added and the mixture was stirred for another 20 min at room temperature. After centrifugation at 25000×*g* at 4 °C for 30 min, the precipitate of the gold-labelled MAb was resuspended with 10 mmol/L PBS (pH 7.4) containing 4% (w/v) polyethylene glycol (PEG)-2000 and 0.1% (w/v) sodium azide and centrifuged again. The precipitate was then resuspended with 5 ml of 10 mmol/L PBS (pH 7.4) containing 2% (w/v) BSA and 0.1% (w/v) sodium azide and stored at 4 °C for use.

2.10.2 CGIA development

CGIA was developed by using the similar method as described by Wang *et al*. (2007). Briefly, the sample absorbent and the conjugate pads were treated with PBS (20 mmol/L, pH 7.4) containing 2% (w/v) BSA, 2% (w/v) sucrose and 0.1% (w/v) sodium azide, and dried at 37 °C for 3 h. Three factors (sensitivity, specificity, and incubation time of CGIA) were used to determine the optimal immobilization concentrations of streptomycin-BSA conjugate, gold-antibody conjugate, and goat anti-mouse antibodies. At optimal conditions, streptomycin-BSA (0.21 mg/ml) and goat anti-mouse antibodies (1 mg/ml) were dispensed onto the nitrocellulose membrane of the test and control lines with a Quanti 3000 BioJets attached to a BioDot XYZ-3000 dispensing platform and dried at 37 °C for 3 h. The gold-labelled MAb was dispensed to the treated conjugate pad at a jetting rate of 7 μl/cm and dried. The treated nitrocellulose membrane, the prepared conjugate pad, the sample pad and the absorbent pad were assembled as immunochromatographic strip. The assembled plate was cut into strips (60 mm×4 mm) with an AZCON Sur-Size automatic guillotine cutter.

2.10.3 CGIA procedure

CGIA was based on the competitive reaction principle and it took 3–5 min to complete a test. When three drops (about 100 μl) of samples with pH adjusted to 6–8 were dropped into the sample absorbent pad and the sample migrated to the conjugate pad, the colloid gold-labelled MAb on the conjugate pad was solubilized and began to migrate along with the sample up to the nitrocellulose membrane. The colloid gold-labelled MAb was captured by the streptomycin-BSA conjugate immobilized on the membrane to display a clear red test line, and the excess colloid gold-labelled MAb migrated farther and was captured by the goat anti-mouse antibodies to display a red control line. If (dihydro)streptomycin exists in the sample, it will

compete with the immobilized streptomycin-BSA conjugate on the test line to bind the limited amount of the colloid gold-labelled MAb. The more (dihydro) streptomycin that exists in the sample, the weaker color observed at the test line. The color intensities will decrease with increasing (dihydro)streptomycin concentrations in the samples. When (dihydro)streptomycin exists in a sufficient amount, it will completely prevent the binding of the colloid gold-labelled MAb with the immobilized streptomycin-BSA conjugate on the test line and there is no visible test line, only a red control line. If the control line has no color, the assay should be considered non-valid. The raw milk or swine urine samples need no pretreatment for the CGIA developed in this study.

3 Results

3.1 Preparation of streptomycin-BSA and streptomycin-OVA conjugates

Streptomycin molecules possess a reactive aldehyde group, which can react with the amine group of carboxymethoxylamine and product a streptomycin derivative containing a reactive carboxyl moiety in the molecule. The carboxyl moiety of streptomycin derivative can react with the amine group of carrier proteins, such that the derivative can couple with carrier proteins (Schnappinger *et al*., 1993; Watanabe *et al*., 2002; Samsonova *et al*., 2005). In this study, the preparation of antigens used the above method and ultraviolet scan analyses found that scan curves of streptomycin-BSA and streptomycin-OVA conjugates obviously differed from those of the streptomycin, BSA, and OVA (Fig. 1). This result indicated that streptomycin was successfully conjugated with carrier protein, BSA, and OVA, respectively.

3.2 Hybridoma production and monoclonal antibody characterization

Streptomycin-BSA conjugate was used as immunogen for the immunization of mice, while streptomycin-OVA conjugate was coated onto ELISA plates to determine the titer and inhibition level of antibodies. Five of the eight mice immunized with streptomycin-BSA produced high titer (1:10000) antisera against streptomycin and were chosen for hybridoma production. In the five cell fusion experiments, hybridomas were obtained in 100% of the initial culture plate wells. Screened by an indirect ELISA, 260 of all the culture plate wells containing hybridomas were positive. Hybridomas showing a strong response were further assayed with an indirect competitive ELISA for determining their ability to recognize un-conjugate streptomycin and their sensitivity. After MAb sensitivity detection and cell cloning, three hybridomas (2A10, 8G3 and 13H5) with the ability to secrete anti-streptomycin antibody were obtained. The most sensitive MAb named 13H5 showed the IC_{50} value of 4.65 ng/ml and the IC_{20} value of 0.21 ng/ml in PBS buffer. Using a mouse MAb isotyping kit, 13H5 was determined to be the subclass IgG_1 with a kappa light chain. The titres of the hybridoma supernatant and ascitic fluid determined

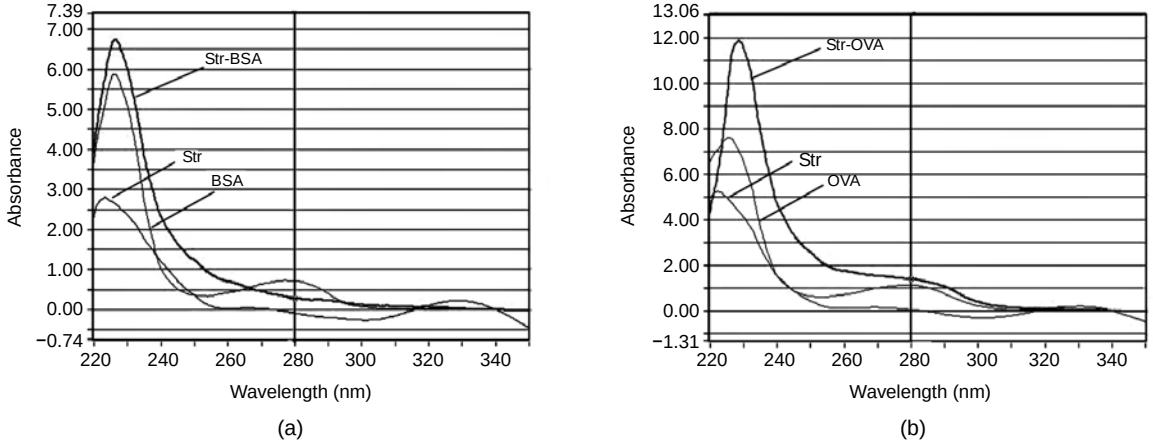

Fig. 1 Ultraviolet scan analyses of streptomycin-BSA (a) and streptomycin-OVA (b) conjugates
Str: streptomycin; Str-BSA: streptomycin-BSA conjugate; Str-OVA: streptomycin-OVA conjugate

by indirect ELISA were 1:3000 and 1:500000, respectively. The IgG was purified from ascites fluid containing MAb with a protein-G affinity column and the IgG yield of purified MAb was 8.63 mg/ml. The purified MAb was stored at −80 °C and used for further evaluation of MAb specificity and subsequent immunoassay development.

3.3 Cross-reactivity of MAb

The MAb specificity was evaluated with the indirect competitive ELISA using different antibiotics as competitors. As shown in Table 1, the MAb has high cross-reactivity with dihydrostreptomycin (118.32%) and negligible cross-reactivity (<0.01) with other antibiotics (neomycin, gentamycin, kanamycin, sulfamethazine, penicillin G, and chlortetracycline). This demonstrated that the MAb is highly specific for dihydrostreptomycin and streptomycin. The MAb has high cross-reactivity with dihydrostreptomycin because they have very similar structure and dihydrostreptomycin contains hydroxyl-methyl group instead of aldehyde group of streptomycin. So, all reported anti-streptomycin antibodies showed a high cross-reactivity (from 75% to 150%) between streptomycin and dihydrostreptomycin (Hammer *et al*., 1993; Schnappinger *et al*., 1993; Haasnoot *et al*., 1999; Baxter *et al*., 2001; Ferguson *et al*., 2002; Watanabe *et al*., 2002; Abuknesha and Luk, 2005; Samsonova *et al*., 2005).

Table 1 Cross-reactivity of the anti-streptomycin MAb with different antibiotics

Antibiotics	IC_{50} (ng/ml)	Cross-reactivity (%)
Streptomycin sulfate	4.65	100.00
Dihydrostreptomycin	3.93	118.32
Neomycin	>50000	<0.01
Gentamycin	>50000	<0.01
Kanamycin	>50000	<0.01
Sulfamethazine	>50000	<0.01
Penicillin G	>50000	<0.01
Chlortetracycline	>50000	<0.01

3.4 Indirect competitive ELISA

From bidimensional titration assays, optimum conditions of the indirect competitive ELISA were selected as follows: optimum concentrations of coating antigen, MAb and HRP-labelled goat anti-mouse IgG for milk and swine urine samples were 3.213, 0.002 and 0.001 μg/ml, respectively. The optimum incubation conditions of the assay were found to be overnight at 4 °C for antigen coating, 1 h at 37 °C for the MAb with samples and HRP-labelled goat anti-mouse IgG, 15 min at 37 °C for substrate color development. Under optimum conditions, the calibration curves were obtained and the measurement range of the streptomycin calibration curve (from IC_{92} to IC_{20}) was between 0.4 and 200 ng/ml, or 0.38 and 197 ng/ml, in the milk or swine urine matrix, respectively (Fig. 2). On the basis of the 5-fold dilutions of samples, the minimum detection limit was 2.0 and 1.9 ng/ml for milk and swine urine samples, respectively.

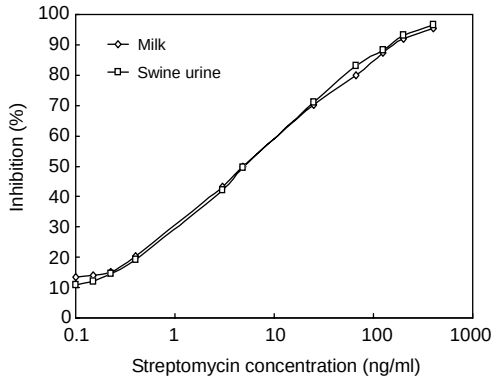

Fig. 2 Mean calibration curves of the ELISA in the two matrices
Data represent the means of four determinations

For milk and swine urine sample analyses, the centrifugation is always used to defat or remove the precipitate and it can reduce matrix effect. Our investigations showed that preliminary centrifugation was necessary for milk and not for swine urine (data not shown).

3.5 Analysis of spiked samples with ELISA

To validate the developed ELISA, milk and swine urine samples were spiked with various amounts of streptomycin and analyzed by the indirect competitive ELISA. Based on the value obtained from the calibration curve, recoveries of streptomycin from the milk and swine urine spiked with different amount of streptomycin (10, 50, 100 and 200 ng/ml) assayed directly with indirect competitive ELISA, were ranged from 81.9% to 105.5% and from 84.3% to 92.2%, respectively (Table 2). The result indicated

the recoveries of the ELISA are sufficient for analyzing streptomycin residues in milk and swine urine samples.

Table 2 Recoveries of streptomycin from spiked milk and swine urine (n=8)

Samples	Level added (ng/ml)	Level found* (ng/ml)	Recovery (%)
Milk	10	9.2±1.3	92.0
	50	49.1±2.9	98.2
	100	105.5±2.1	105.5
	200	163.8±0.9	81.9
Swine urine	10	8.6±2.6	86.0
	50	45.8±3.0	91.6
	100	92.2±1.0	92.2
	200	168.5±3.1	84.3

* Mean±SD

3.6 CGIA

The CGIA conditions were optimized for high sensitivity, but without any nonspecific bindings. The positive samples were visually judged by a weaker colour test line compared with control line or no color test line, and the negative samples were judged by a same color or a stronger colour test line compared with control line. Streptomycin-free milk and swine urine spiked with streptomycin (0, 20, 30, 40, 50, 60 and 70 ng/ml) were detected with the CGIA to estimate the reliability of the assay. The red color of the test line on the strip gradually thinned with an increasing amount of streptomycin and completely disappeared at 50 ng/ml of streptomycin in milk or swine urine sample (Fig. 3). The lower detection limit of streptomycin of the assay is defined as the least concentration of streptomycin in the sample at which the color of the test line is weaker than that of the control line. As shown in Fig. 3, 20 ng/ml of streptomycin caused a slight but distinguishable degressive color intensity compared with the control line with naked eyes. Thus, 20 ng/ml of streptomycin in PBS, milk, and swine urine samples was defined to be a lower detection limit of the CGIA, far below 200 ng/ml of the European Union (EU) maximum residue limit (MRL) for streptomycin in milk. Milk and swine urine spiked with dihydrostreptomycin (0, 20, 30, 40, 50, 60 and 70 ng/ml) were also detected with the CGIA and the results were the same as those of milk and swine urine spiked with streptomycin (data not shown). To analyze the specificity of the CGIA, 10000 ng/ml of neomycin, gentamycin, kanamycin, sulfamethazine, penicillin G and chlortetracycline in PBS were detected with the CGIA. The detection results of all those antibiotics at 10000 ng/ml concentration were negative, which indicated the assay is specific for streptomycin (Fig. 4). Therefore, the developed CGIA is suitable for the screening of streptomycin residues in milk and swine urine samples.

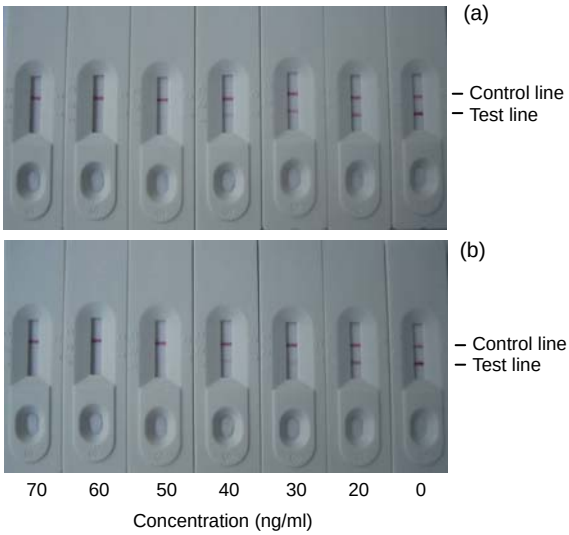

Fig. 3 Detection of streptomycin with the CGIA
A series of dilutions (0, 20, 30, 40, 50, 60 and 70 ng/ml) of streptomycin were prepared in streptomycin-free milk (a) and swine urine (b)

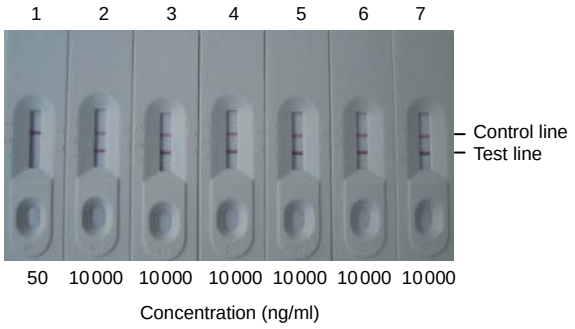

Fig. 4 Specificity analysis of the CGIA
1, 2, 3, 4, 5, 6 and 7 were streptomycin, neomycin, gentamycin, kanamycin, sulfamethazine, penicillin G and chlortetracycline, respectively

3.7 Applications of CGIA and indirect competitive ELISA in milk and swine urine samples

Streptomycin residues in milk and swine urine samples collected from Hangzhou nearby farms or

markets were detected with the CGIA. Among the 86 milk and 41 swine urine samples, 1 milk sample and 1 swine urine sample were shown to contain streptomycin residues. The positive samples can be confirmed by the indirect competitive ELISA. On the basis of the streptomycin calibration curve of the indirect competitive ELISA, the concentrations of the two positive samples were 30.5 and 62.8 ng/ml, respectively.

4 Discussion

In the present study, an MAb 13H5 specific for the common structure of the streptomycin and dihydrostreptomycin was prepared and showed no cross-reactivity with the other tested antibiotics, such as neomycin, gentamycin, kanamycin, sulfamethazine, penicillin G, and chlortetracycline. Using the MAb, a highly sensitive and specific ELISA and CGIA for detection of streptomycin residues in milk and swine urine samples have been successfully developed. The analyses of spiked samples proved that the two immunoassays are suitable to detect streptomycin residues in milk and swine urine samples.

Some groups had developed ELISA and CGIA to analyze streptomycin residues in animal urine and tissue samples (Schnappinger *et al*., 1993; Heering *et al*., 1998; Haasnoot *et al*., 1999; Verheijen *et al*., 2000; Watanabe *et al*., 2002; Abuknesha and Luk, 2005; Samsonova *et al*., 2005). Because of high specificity and affinity of the prepared MAb, the two assays based on MAb for streptomycin detection were shown to be highly sensitive and specific. The lower detection limits of the developed competitive ELISA for milk and swine urine samples were 2.0 and 1.9 ng/ml, respectively. The lower detection limits of the developed CGIA for milk and swine urine samples were 20.0 ng/ml. To our knowledge, no publications about immunoassay to detect streptomycin residues in food have reported such a lower detection limit of the developed method as described in this study.

In this study, the described CGIA is easy to perform and the result can be shown within 3–5 min without any equipment, and all needed reagents were included in the strip. The result of CGIA is visual and it can be used as a convenient qualitative or semi-quantitative method for the rapid screening of milk and swine urine samples for the presence of streptomycin residues above 20 ng/ml of the detection limit, which is far lower to EU MRL level of 200 ng/ml. So, the assay is especially suitable for on-site screening of milk or swine urine sample, for example, on farms and in swine slaughterhouses.

It is likely that the two assays can be eventually used to efficiently and accurately detect (dihydro) streptomycin residues in other animal food products, such as bee honey, fishes, chicken and eggs.

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