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Development of an ultrasensitive ic-ELISA and immunochromatographic strip assay for the simultaneous detection of florfenicol and thiamphenicol in eggs

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ABSTRACT

An ultrasensitive monoclonal antibody-based gold nanoparticle immunochromatographic strip assay and indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) were developed to detect florfenicol (FF) and thiamphenicol (TAP) in egg samples. The ic-ELISA, with optimized pH, methanol content and sodium chloride content, exhibited an IC₅₀ value of 0.2 ng/mL for FF and 0.27 ng/mL for TAP, with the working range of 0.05–0.77 and 0.05–1.42 ng/mL, respectively. The optimized ic-ELISA showed negligible cross-reactivity with other phenols and broad-spectrum antibiotics. The recoveries in egg samples using the ic-ELISA ranged from 84% to 115% with a coefficient of variation of less than 5%. Based on this monoclonal antibody, a rapid and ultrasensitive immunochromatographic strip assay was developed with a cutoff value of 1 ng/mL for FF and TAP. Our results indicated that both developed methods were highly useful for screening FF and TAP in eggs.

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KEYWORDS

Florfenicol; ic-ELISA; immunochromatographic strip assay; monoclonal antibody; thiamphenicol

Introduction

Chloramphenicol (CAP) is considered a representative drug of the amphenicol family of antibiotics and has been used as a treatment and a prophylactic in food-producing animals for a long time. However, CAP has resulted in aplastic anaemia and other potentially fatal side effects in humans (Fodey et al., 2013). Florfenicol (FF) and thiamphenicol (TAP), which show high antibacterial activity, are new desirable substitutes for CAP with similar structural formulas. Compared to CAP, TAP and FF appear to be less toxic and have been widely used in veterinary clinical practice to treat disease and in the prophylaxis of bacterial infections (Hu et al., 2014). However, TAP and FF have been reported to have adverse effects on human health, such as the potential for the emergence of drug-resistant bacteria, immune dysfunction and drug residues in food-animal tissues (Guan et al., 2011). Consequently, the use of FF and TAP is strictly limited in China and in many other countries. In the EU, the maximum residue limit (MRL) for TAP was set at 50 and 100–3000 µg/kg for FF in the target tissues of food-producing species (European Commission, 2010).

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Currently, the main methods of detecting TAP and FF are various instrumental analytical methods, including high performance liquid chromatography (HPLC) (Xie et al., 2011), gas chromatography (Pfenning et al., 1997), liquid chromatography-tandem mass spectrometry (Rezende, Filho, & Rocha, 2012; Tao, Zhu, et al., 2014), and gas chromatography-mass spectrometry (Yikilmaz & Filazi, 2015). These methods have obvious advantages in terms of sensitivity and accuracy, but they require specific instrumentation and an operator. Furthermore, a lot of time is needed for sample preparation. Thus, these methods are not applicable in the determination of numerous samples.

Immunological methods, based on the specific binding reaction of an antibody and antigen, have rapidly been developed for screening food safety. Enzyme-linked immunosorbent assay (ELISA) and immunochromatographic strip (ICS) assay have been successfully used to monitor many drug residues due to their rapidity, user-friendliness, and high sensitivity (Samsonova, Cannavan, & Elliott, 2012; Tao, Yu, et al., 2014; Xu, Xu, Ma, Kuang, & Xu, 2015; Yao, Liu, Song, Kuang, & Xu, 2017). Luo et al. produced an ELISA specifically for FF detection in fish feed based on a monoclonal antibody (mAb) with an IC_{50} of 2.5 ng/mL (2009). Guo's group prepared a mAb and developed ELISAs and ICSs for the detection of CAP, TAP and FF in milk and honey with an IC_{50} of 0.39 ng/mL for TAP and cross-reactivity (CR) of 15.6% for FF (Guo, Song, et al., 2015).

In the present study, we prepared an ultrasensitive mAb for TAP and FF. Based on this antibody, we developed both an ultrasensitive ICS assay and indirect competitive ELISA (ic-ELISA) for the simultaneous detection of FF and TAP in eggs.

Materials and methods

Reagents and materials

The standards of FF, TAP, CAP and FFA were purchased from J&K Scientific Ltd. (Beijing, China). Bovine serum albumin (BSA), ovalbumin (OVA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), succinic anhydride, complete Freund's adjuvant (FCA), incomplete Freund's adjuvant (FIA), 3,3',5,5'-tetramethylbenzidine (TMB), gelatin and Tween-20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Enzyme immunoassay grade horseradish peroxidase (HRP)-labeled goat anti-mouse immunoglobulin was obtained from Kangcheng Bioengineering Co. (Shanghai, China). Reagents for cell fusion and culture (e.g. polyethylene glycol 1500 solution, HAT supplement, HT medium supplement, and 1640 cell culture medium) were obtained from Life Technologies Co., Ltd. (Shanghai, China).

The materials for ICS production, including glass fibre membrane, NC membrane, H5076 filter paper and Ahlstrom 8964, were purchased from JieYi Biotech Co., Ltd. (Shanghai, China).

All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Solutions

The coating buffer (CB, 0.05 M, pH 9.6) was prepared by dissolving 2.93 g of $NaHCO_3$ and 1.59 g of Na_2CO_3 in 1 L of purified water. The blocking buffer was added to 0.2% gelatin in

the CB. The assay buffer was phosphate buffer solution (PBS, 0.01 M, pH 7.4), the washing buffer (PBST) contained 0.05% Tween-20 (v/v) in 0.01 M PBS. The substrate solution consisted of solutions A (Na_2HPO_4 , citric acid and H_2O_2) and B (0.06% v/v TMB in glycol) at the ratio of 5:1 (v/v). The stop solution was 2 M sulphuric acid.

Synthesis of hapten FF-HS

The hapten FF-HS, which involved coupling FF with succinic anhydride, was synthesized in our laboratory following the method described by Hao, Guo, and Xu (2006). Specifically, FF (1.07 g, 3 mmol), succinic anhydride (0.6 g, 6 mmol) and pyridine (50 mL) were mixed and heated at 60°C for 4 h in a reflux condenser. The reaction mixture was evaporated using a rotary evaporator. The residue was re-dissolved with ethyl acetate (20 mL), and then 1 mol/L HCl (20 mL) and purified water were added and extracted three times. A pink solid hapten was obtained after drying in vacuum and verified by mass spectrum.

Preparation of immunogen and coating antigen

Synthesis of immunogen FF-HS-MA-BSA

The immunogen was prepared by linking the hapten to BSA using the mixed-anhydride reaction (Xu, Xu, Ma, Kuang, et al., 2015). Firstly, 15 mg FF-HS was dissolved in 1 mL of *N,N*-dimethylformamide and left at 4°C for 15 min. Subsequently, 8.9 μL of tributylamine and 4.6 μL of isobutyl-chloroformate were added to the solution with stirring. The solution was mixed at 4°C for 1 h (solution A). In addition, 40 mg of BSA was dissolved in 4 mL of CB and cooled to 4°C (solution B). Solution A was added dropwise to solution B with constant stirring in ice water, and reacted for 4 h. Finally, the solution was dialysed against PBS for 3 days at room temperature, and identified by UV and polyacrylamide gel electrophoreses.

Synthesis of coating antigen FF-HS-EDC-OVA/BSA

The coating antigen was prepared by coupling FF-HS with OVA (or BSA) using an active ester method (Gu, Liu, Song, Kuang, & Xu, 2016; Suryoprabowo, Liu, Peng, Kuang, & Xu, 2015). Fifteen milligrams of FF-HS, 18 mg of EDC, and 11 mg of NHS were dissolved in 2 mL of 50% *N,N*-dimethylformamide, and the mixture was stirred for 4 h at room temperature (solution A). In addition, 40 mg of OVA (or BSA) was dissolved in 2 mL of CB, respectively (solution B). Solution A was added dropwise to solution B with constant stirring, and incubated overnight at room temperature. The solution was dialysed and identified following the method described by Xing, Hao, Liu, Xu, and Kuang (2014).

Preparation of monoclonal antibody

Female BALB/c mice (aged 6–8 weeks) were immunized with 0.1 mL of immunogen per mouse by subcutaneous multisite injections (Liu, Song, et al., 2014; Xing et al., 2015). The mice was immunized firstly using a 1:1 mixture emulsion of FF-HS-MA-BSA (0.1 mg) and complete Freund adjuvant, and then booster immunizations were

given over a period of 3 weeks by injecting a 1:1 mixture emulsion of FF-HS-MA-BSA (0.05 mg) and incomplete Freund adjuvant (Liu, Kuang, Peng, Wang, & Xu, 2014). After the third immunization, blood samples were collected a week after each booster and examined by ic-ELISA. Positive BALB/c mice with the highest inhibition were given the final immunization with 25 µg of FF-HS-MA-BSA by intraperitoneal injection. After 21 days, the mice were used for cell fusion. After subcloning and selection, the eligible cell line continued to expand and was injected into BALB/c mice to produce ascites. The ascites were purified by caprylic acid and ammonium sulphate precipitation, and then dialysed against PBS for 72 h at 4°C. The subtypes of the purified mAb were identified using a mouse mAb subtyping kit.

Indirect competitive ELISA

The indirect immunoassay was performed in 96-well polystyrene titration plates as described earlier, with the aim of determining the concentrations of the antibodies and coating antigens (Guo, Liu, et al., 2015; Liu, Xing, Yan, Kuang, & Xu, 2014). Briefly, a microtiter plate was coated with antigen conjugates (100 µL/well) in coating solution and incubated for 2 h at 37°C. After washing three times with PBST, 200 µL/well blocking buffer was added to the plates and incubated for 2 h at 37°C. Following removal of the blocking solution, the optimal antibody dilution (50 µL) and standard (50 µL) were added and incubated for 0.5 h at 37°C. Then after three washes, 100 µL/well of goat anti-mouse HRP (1:3000 in PBST with gelatin) was added and incubated for 0.5 h at 37°C. The plates were washed with PBST and incubated with 100 µL of substrate solution for 15 min at 37°C. Finally, 50 µL/well of the stop solution was added to halt colour enhancement, and the optical density (OD) value was measured by micro-plate detector at 450 nm.

Furthermore, in order to study the assay performance of ic-ELISA and improve immunoassay sensitivity, several experimental factors, including organic solvents (0, 10%, 20%, 30% and 40%), pH (5.5, 6.5, 7.5, 8.5 and 9.5), and ionic strength (0, 0.4%, 0.8%, 1.6% and 3.2% NaCl), were evaluated and optimized. Optimal values were judged by the OD value of zero concentration and IC₅₀.

Cross-reactivity

The specificity of the mAb was assessed by the cross-reactivity value using several structurally related antibiotics (TAP, CAP and FF amine). The IC₅₀ value of each analogue was determined using the optimized ic-ELISA procedure described above. The CR values were evaluated based on the following formula: CR (%) = (IC₅₀ of FF/IC₅₀ of the tested analogues) × 100% (Kong, Liu, Song, Kuang, & Xu, 2016).

Recovery in egg samples using ic-ELISA

Negative egg samples were added to the standards of TAP and FF at different concentrations, and mixed by a homogenizer to ensure uniformity of the sample; 2.5 g of the spiked egg samples was extracted with 10 mL of anhydrous acetonitrile. After the sample had been rotated for 5 min and centrifuged for 5 min at 4500×g, 4 mL of the

upper organic solution was removed, placed in a glass tube and evaporated under nitrogen flow at 50–60°C. The residue was dissolved in 1 mL of *n*-hexane and 1 mL of PBS (0.01 mol/L, pH 7.4) at 1-min intervals under vortex. The mixture was centrifuged at 5000×g for 5 min, and the solution was analysed (An et al., 2016).

Preparation of colloidal gold nanoparticles

The protocol used for colloidal gold nanoparticles synthesis was designed based on that described previously (Lou, Ye, Li, & Wu, 2012; Sun et al., 2012). Briefly, 50 mL H₂AuCl₄·4H₂O (0.01%, m/v) solution was heated to boiling with constant stirring in a clean flask. Then, 1 mL of freshly prepared sodium citrate solution (1%, w/v) was quickly added under stirring and constant boiling for 6 min while the claret colour appeared. The solution was then cooled to room temperature and stored in the refrigerator at 4°C. The uniformity of colloidal gold nanoparticle diameter was characterized by transmission electron microscopy.

Combining the mAb with colloidal gold particles

The purified mAb was combined with colloidal gold particles based on the modified experimental scheme of Hao et al. (2012) and Liu, Luo, et al., (2014). 0.1 M K₂CO₃ was added to the colloidal gold particle solution (10 mL) to adjust the pH to 8.0, and then the purified mAb (2 g/L, 80 μL) was added slowly and mixed for 1 h at ambient temperature. Then, 50 mL of 10% (m/v) BSA was added to block any unbound sites. After 2 h incubation, the mixture was centrifuged at 8000×g for 45 min to remove excess reagents. Finally, the residue was re-suspended in re-suspension buffer (0.002 M BB, 0.01% Tween-20, 1% sucrose, 0.1% PEG, 1% mannitol, and 0.04% NaN₃, pH 7.2) and stored at 4°C.

Fabrication and principle of the ICS assay

The ICS structure was similar to that described in previous studies (Wang et al., 2011; Zou, Cui, Liu, Song, & Kuang, 2017). The NC membrane was loaded on the centre of the support plate. The control and test lines were then sprayed onto the NC membrane with 0.5 mg/mL of goat anti-mouse IgG and 1 mg/mL of the coating antigen FF-BSA, respectively, and dried for 2 h at 37°C. The absorbent pads were pasted on the sides of the support plate close to the control line, with a sample pad on the other side. Finally, the fabricated plate was cut into 2.5-mm wide strips.

The sample solution was reacted with the gold-labelled mAb solution for 5 min at ambient temperature. When the strip was inserted, the mixture migrated from the sample pad to the absorbent pad by the capillary effect (Xu, Xu, Ma, Liu, et al., 2015). The gold-labelled mAb was combined with FF or TAP in the sample and the coating antigen loaded on the test line competitively. Therefore, a little of the labelled mAb conjugate with coating antigen was on the test line when a high concentration of FF or TAP was present in the sample solution, resulting in a weak colour or no colour on the test line. In contrast, a stronger colour come into being on the test line when a low concentration of FF or TAP was present. The control line appeared red in each test, and this was because

labelled mAb can react with goat anti-mouse IgG regardless of the presence of FF or TAP in the sample solution.

Characteristics of the ICS assay

Two milligrams of FF was dissolved in 2 mL of *N,N*-dimethyl formamide as a standard stock solution (1 mg/mL), and the stock solution was then diluted to a series of concentrations with PBS (0, 0.05, 0.1, 0.25, 0.5 and 1 ng/mL), and TAP was similarly diluted to 0, 0.1, 0.25, 0.5 and 1 ng/mL. Subsequently, 50 μ L of each standard dilution was mixed with the labelled mAb in the micro-well plate for 5 min, and the sample pad of the ICS was inserted into the mixture, and the results were analyzed based on the colour change after 8 min.

Determination of TAP and FF in eggs using ICS

Negative egg samples were used for ICS analysis. Analogue standards were added to the egg samples, which were treated in accordance with the recovery test for ic-ELISA.

Results and discussion

Antigen characterization

FF has weak immunogenicity due to a low molecular weight, and must be attached to carrier proteins (BSA or OVA). In order to derive a carboxyl used to react with the protein, FF-HS was obtained by a series of reactions with succinic anhydride. In addition, FF-HS has a long extra carbon chain, which makes the functional group of FF more exposed.

In this study, FF-HS was covalently attached to BSA and OVA by the mixed-anhydride reaction and the active ester method, respectively. The conjugations were identified by UV spectra. As shown in [Figure 1](#), FF-HS had two obvious absorption peaks at 267 and 273 nm, and carrier proteins (BSA and OVA) had an absorption peak at 280 nm. The UV spectra of conjugated antigen had a distinct characteristic peak at 267–280 nm, covering the peaks of FF-HS and the carrier proteins. These results indicated that the immunogen and coating antigen were successfully coupled.

Optimization of ic-ELISA

The coating of FF-HS-EDC-OVA and FF-HS-EDC-BSA were compared using ic-ELISA, and FF-HS-EDC-BSA was selected for the following research based on a higher maximum absorbance value (Abs_{max}) and lower IC_{50} value. In this optimization experiment, the concentration of coating and mAb 1G7 were 0.15 and 0.025 μ g/mL, respectively. The influence of pH, NaCl content and methanol content in PBS are shown in [Figure 2](#). As shown in [Figure 2\(a\)](#), the OD value gradually decreased with increased NaCl content in PBS. An NaCl content of 1.6% was chosen based on the lowest IC_{50} value and suitable Abs_{max} . [Figure 2\(b\)](#) shows a small difference with different pH values, pH 7.5 resulted in the biggest ratio value of A_{max}/IC_{50} . As shown in [Figure 2\(c\)](#), methanol content in PBS had little effect on Abs_{max} but the IC_{50} value was lower in the 0% condition. From the above results, the optimum values of the three factors in ic-ELISA were pH 7.4, 1.6% NaCl and 0% methanol in PBS. The standard curve was created based on optimized

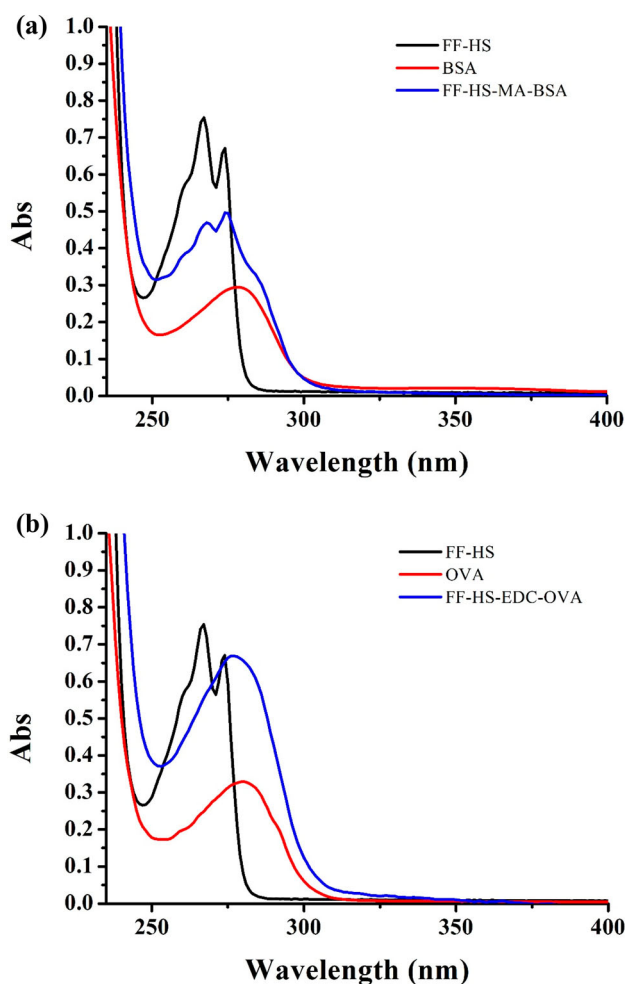


Figure 1. The ultraviolet-visible absorption spectra of antigen. (a) Immunogen FF-HS-MA-BSA; (b) coating antigen FF-HS-EDC-OVA.

conditions (Figure 2(d)), and IC_{50} values were 0.2 ng/mL for FF and 0.27 ng/mL for TAP, and the limit of detection (LOD) was 0.053 ng/mL.

Cross-reactivity

Under the optimized ic-ELISA, the antibody specificity was analysed by measuring IC_{50} and CR with the amphenicol family of antibiotics (Table 1). The produced antibody had good recognition for FF, TAP and FF-HS, and the CR value with other analogues was <0.5%. Thus, the antibody was specific for FF and TAP.

Recovery test in egg samples using ic-ELISA

Negative egg samples were spiked with FF and TAP, resulting in concentrations ranging from 0.1 to 1 ng/g, respectively. After the spiked samples were pretreated, the recovery rate

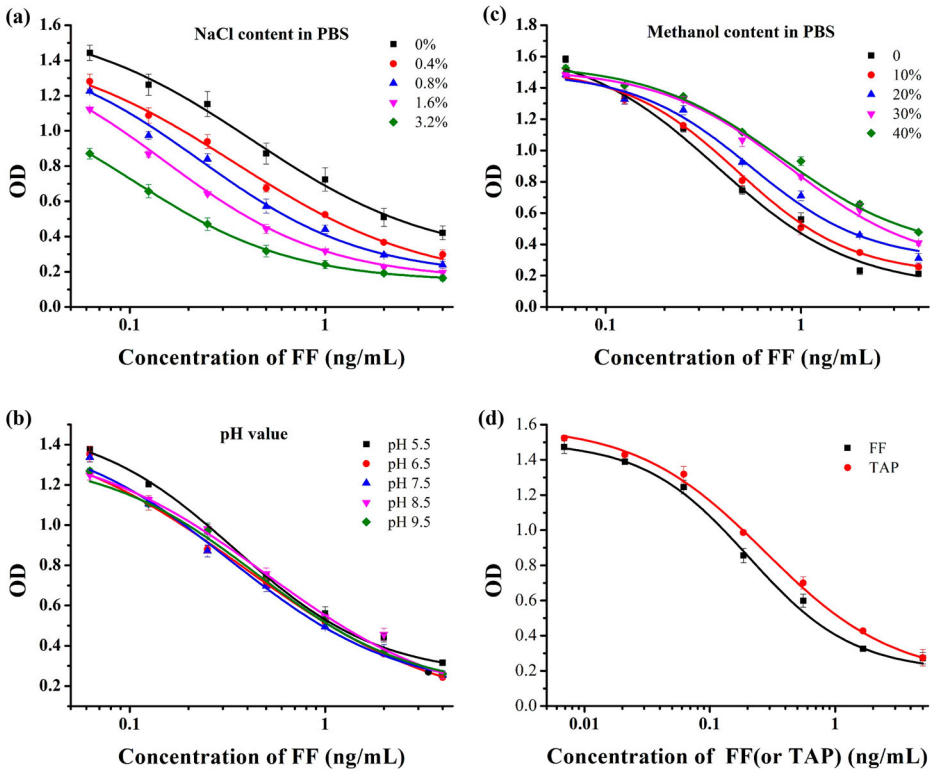


Figure 2. Optimization of assay buffer for ic-ELISA. (a) The influence of NaCl content in PBS; (b) the influence of pH value; (c) the influence of methanol content in PBS; (d) standard inhibition curve for FF and TAP in the optimized conditions.

was calculated by the OD of the samples and the standard inhibition curve. Recovery was 86–115% for FF and 84–109% for TAP, the coefficients of variation were all less than 5% (Table 2).

Characterization and optimization of the ICS

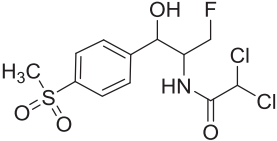
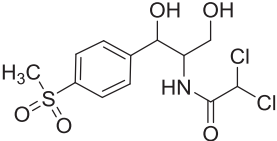
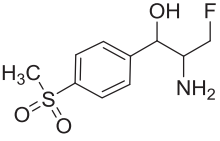
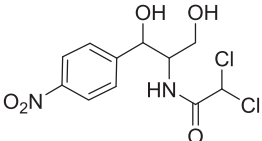
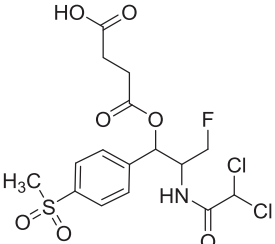
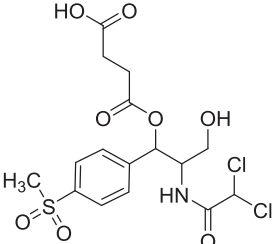
The ICS was prepared and tested by the wet method. In this study, 13 surfactants, added to the suspension (Figure 3), were used for optimization in order to obtain a good capillarity effect, and 5% BSA was selected for subsequent experiments.

Standards of FF and TAP were diluted with PBS to form a concentration gradient of spiked solutions. The test strip showed a red band at the control line at all times, while the red colour of the test line decreased or disappeared with an increase in the standard concentration. Figure 4 shows that the cut-off value was 1 ng/mL for FF and 1 ng/mL for TAP.

Analysis of egg samples using ICS

The detection of real samples was deemed to be a key factor in the evaluation of the test strip. The spiked egg samples were prepared with FF (0, 0.1, 0.25, 0.5, 1 and 2 ng/mL) and

Table 1. CR of the mAb with FF, TAP and other related compounds.

Chemical	Structure	IC ₅₀ (ng/mL)	CR (%)
FF		0.2	100
TAP		0.27	74
FFA		>1000	<1
CAP		>50	<1
FF-HS		0.18	111.1
TAP-HS		1.4	14.3

TAP (0, 0.2, 0.5, 1 and 2 ng/mL). The results were observed visually (Figure 5), and the cut-off values of FF and TAP were all 0.5 ng/mL. Thus, the ICS was useful for the detection of FF and TAP residues in egg samples.

Table 2. Recovery rates of FF and TAP in egg samples.

Additive	Spiked level (ng/g)	Mean (ng/g) ± SD	Recovery rate (%)	CV (%)
FF	0.1	0.087 ± 0.003	87	2.9
	0.2	0.172 ± 0.002	86.1	1.3
	0.5	0.564 ± 0.012	112.7	2.1
	1	1.152 ± 0.052	115.2	4.5
TAP	0.1	0.09 ± 0.003	90.6	3.7
	0.2	0.17 ± 0.007	86.5	4.3
	0.5	0.42 ± 0.005	84.2	1.1
	1	1.09 ± 0.028	108.9	2.6

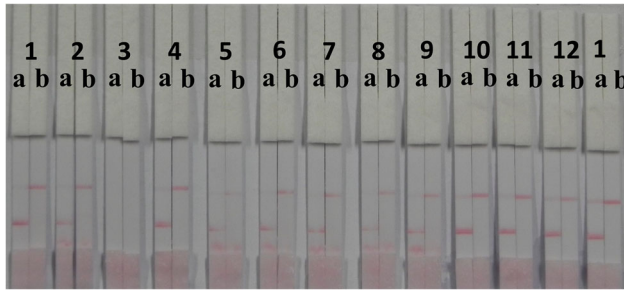


Figure 3. Optimization of surfactants. (1) Casein, (2) suspension buffer, (3) polyvinyl alcohol, (4) sucrose, (5) polyvinyl pyrrolidone, (6) trehalose, (7) Brij-35, (8) Triton X-100, (9) Sorbitol, (10) BSA, (11) Tween-20, (12) Mannitol, and (13) Polyethylene glycol. a = blank; b = 5 ng/mL of FF.

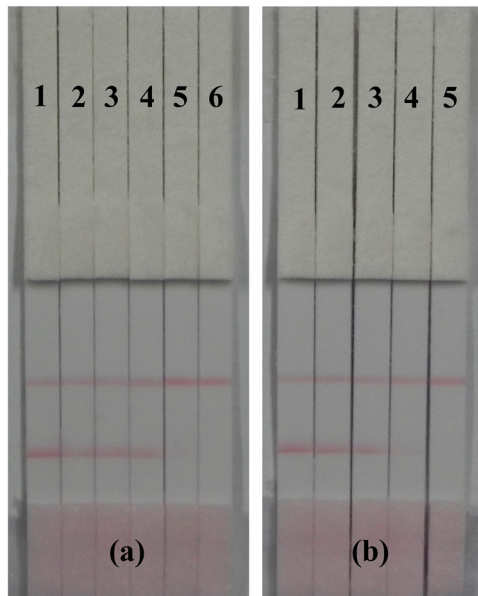


Figure 4. Image of the detection of a series concentration of standard in PBS. (a) FF: 1 = 0 ng/mL, 2 = 0.05 ng/mL, 3 = 0.1 ng/mL, 4 = 0.25 ng/mL, 5 = 0.5 ng/mL, 6 = 1 ng/mL; (b) TAP: 1 = 0 ng/mL, 2 = 0.1 ng/mL, 3 = 0.25 ng/mL, 4 = 0.5 ng/mL, 5 = 1 ng/mL.

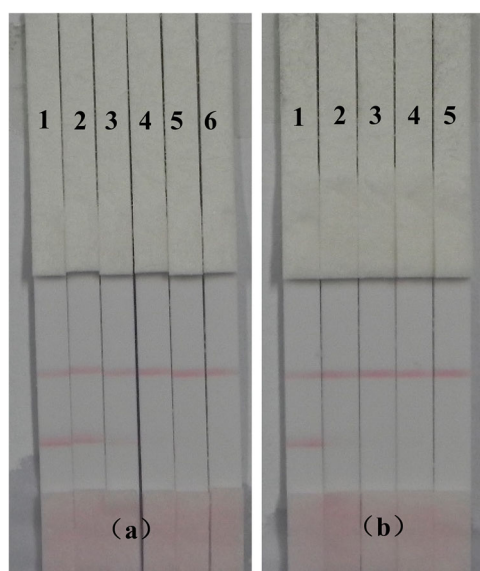


Figure 5. Image of the detection of a series concentration of standard in egg samples. (a) FF: 1 = 0 ng/mL, 2 = 0.1 ng/mL, 3 = 0.25 ng/mL, 4 = 0.5 ng/mL, 5 = 1 ng/mL, 6 = 2 ng/mL; (b) TAP: 1 = 0 ng/mL, 2 = 0.2 ng/mL, 3 = 0.5 ng/mL, 4 = 1 ng/mL, 5 = 2 ng/mL.

Conclusions

In this study, an ultrasensitive mAb was prepared for the simultaneous detection of FF (IC_{50} value of 0.2 ng/mL) and TAP (IC_{50} value of 0.27 ng/mL), with a low CR value with other analogues. By optimizing organic solvents, pH, and ionic strength, the working range of ic-ELISA was 0.053–0.767 ng/mL for FF and 0.053–1.415 ng/mL for TAP; it also had good recovery in egg samples. In addition, we developed a rapid and portable ICS assay, which was highly sensitive for the detection of FF and TAP in egg samples. The ic-ELISA and ICS were not only used for rapid detection in egg samples, but also for other food samples. The use of these techniques is more convenient for monitoring hazardous residues in daily life.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

An, L., Wang, Y., Pan, Y., Tao, Y., Chen, D., Liu, Z., ... Yuan, Z. (2016). Development and validation of a sensitive indirect competitive enzyme-linked immunosorbent assay for the screening of

- florfenicol and thiamphenicol in edible animal tissue and feed. *Food Analytical Methods*, 9(9), 2434–2443.
- European Commission. (2010). Commission regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Off J Eur Union. L*, 15(1).
- Fodey, T. L., George, S. E., Traynor, I. M., Delahaut, P., Kennedy, D. G., Elliott, C. T., & Crooks, S. R. (2013). Approaches for the simultaneous detection of thiamphenicol, florfenicol and florfenicol amine using immunochemical techniques. *Journal of Immunological Methods*, 393(1), 30–37.
- Gu, H., Liu, L., Song, S., Kuang, H., & Xu, C. (2016). Development of an immunochromatographic strip assay for ractopamine detection using an ultrasensitive monoclonal antibody. *Food and Agricultural Immunology*, 27(4), 471–483.
- Guan, S., Lu, J., Shen, X., Qian, W., Liu, J., & Deng, X. (2011). Florfenicol impairs the immune responses to vaccination against foot-and-mouth disease in mice. *Immunopharmacology and Immunotoxicology*, 33(4), 609–613.
- Guo, J., Liu, L., Xue, F., Xing, C., Song, S., Kuang, H., & Xu, C. (2015). Development of a monoclonal antibody-based immunochromatographic strip for cephalexin. *Food and Agricultural Immunology*, 26(2), 282–292.
- Guo, L., Song, S., Liu, L., Peng, J., Kuang, H., & Xu, C. (2015). Comparison of an immunochromatographic strip with ELISA for simultaneous detection of thiamphenicol, florfenicol and chloramphenicol in food samples. *Biomedical Chromatography*, 29(9), 1432–1439.
- Hao, K., Guo, S., & Xu, C. (2006). Development and optimization of an indirect enzyme-linked immunosorbent assay for thiamphenicol. *Analytical Letters*, 39(6), 1087–1100.
- Hao, C., Xua, L., Xing, C., Kuang, H., Wang, L., & Xu, C. (2012). Oligonucleotide-based fluorogenic sensor for simultaneous detection of heavy metal ions. *Biosensors and Bioelectronics*, 36(1), 174–178.
- Hu, D., Zhang, T., Zhang, Z., Wang, G., Wang, F., Qu, Y., ... Liu, S. (2014). Toxicity to the hematopoietic and lymphoid organs of piglets treated with a therapeutic dose of florfenicol. *Veterinary Immunology and Immunopathology*, 162(3), 122–131.
- Kong, D., Liu, L., Song, S., Kuang, H., & Xu, C. (2016). Development of ic-ELISA and lateral-flow immunochromatographic assay strip for the detection of folic acid in energy drinks and milk samples. *Food and Agricultural Immunology*, 27(6), 841–854.
- Liu, L., Kuang, H., Peng, C., Wang, L., & Xu, C. (2014). Fragment-based hapten design and screening of a highly sensitive and specific monoclonal antibody for ractopamine. *Analytical Methods*, 6(1), 229–234.
- Liu, L., Luo, L., Suryoprabowo, S., Peng, J., Kuang, H., & Xu, C. (2014). Development of an immunochromatographic strip test for rapid detection of ciprofloxacin in milk samples. *Sensors*, 14(9), 16785–16798.
- Liu, N., Song, S., Lu, L., Nie, D., Han, Z., Yang, X., ... Zheng, X. (2014). A rabbit monoclonal antibody-based sensitive competitive indirect enzyme-linked immunoassay for rapid detection of chloramphenicol residue. *Food and Agricultural Immunology*, 25(4), 523–534.
- Liu, L., Xing, C., Yan, H., Kuang, H., & Xu, C. (2014). Development of an ELISA and immunochromatographic strip for highly sensitive detection of microcystin-LR. *Sensors*, 14(8), 14672–14685.
- Lou, S., Ye, J.-y., Li, K.-q., & Wu, A. (2012). A gold nanoparticle-based immunochromatographic assay: The influence of nanoparticulate size. *The Analyst*, 137(5), 1174–1181.
- Luo, P., Cao, X., Wang, Z., Jiang, H., Zhang, S., Chen, X., ... Shen, J. (2009). Development of an enzyme-linked immunosorbent assay for the detection of florfenicol in fish feed. *Food and Agricultural Immunology*, 20(1), 57–65.
- Pfenning, A. P., Madson, M. R., Roybal, J. E., Turnipseed, S. B., Gonzales, S. A., Hurlbut, J. A., & Salmon, G. D. (1997). Simultaneous determination of chloramphenicol, florfenicol, and thiamphenicol residues in milk by gas chromatography with electron capture detection. *Journal of AOAC International*, 81(4), 714–720.
- Rezende, D., Filho, N. F., & Rocha, G. (2012). Simultaneous determination of chloramphenicol and florfenicol in liquid milk, milk powder and bovine muscle by LC-MS/MS. *Food Additives & Contaminants: Part A*, 29(4), 559–570.

- Samsonova, J. V., Cannavan, A., & Elliott, C. T. (2012). A critical review of screening methods for the detection of chloramphenicol, thiamphenicol, and florfenicol residues in foodstuffs. *Critical Reviews in Analytical Chemistry*, 42(1), 50–78.
- Sun, F., Liu, L., Ma, W., Xu, C., Wang, L., & Kuang, H. (2012). Rapid on-site determination of melamine in raw milk by an immunochromatographic strip. *International Journal of Food Science & Technology*, 47(7), 1505–1510.
- Suryoprabowo, S., Liu, L., Peng, J., Kuang, H., & Xu, C. (2015). Antibody for the development of specific immunoassays to detect nadifloxacin in chicken muscles. *Food and Agricultural Immunology*, 26(3), 317–324.
- Tao, X., Yu, X., Zhang, D., Shi, W., Jiang, H., Wang, X., ... Shen, J. (2014). Development of a rapid chemiluminescent ciELISA for simultaneous determination of florfenicol and its metabolite florfenicol amine in animal meat products. *Journal of the Science of Food and Agriculture*, 94(2), 301–307.
- Tao, Y., Zhu, F., Chen, D., Wei, H., Pan, Y., Wang, X., ... Yuan, Z. (2014). Evaluation of matrix solid-phase dispersion (MSPD) extraction for multi-fenicol determination in shrimp and fish by liquid chromatography–electrospray ionisation tandem mass spectrometry. *Food Chemistry*, 150, 500–506.
- Wang, L., Ma, W., Chen, W., Liu, L., Ma, W., Zhu, Y., ... Xu, C. (2011). An aptamer-based chromatographic strip assay for sensitive toxin semi-quantitative detection. *Biosensors and Bioelectronics*, 26(6), 3059–3062.
- Xie, K., Jia, L., Yao, Y., Xu, D., Chen, S., Xie, X., ... Wang, J. (2011). Simultaneous determination of thiamphenicol, florfenicol and florfenicol amine in eggs by reversed-phase high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B*, 879(23), 2351–2354.
- Xing, C., Hao, C., Liu, L., Xu, C., & Kuang, H. (2014). A highly sensitive enzyme-linked immunosorbent assay for copper (II) determination in drinking water. *Food and Agricultural Immunology*, 25(3), 432–442.
- Xing, C., Liu, L., Song, S., Feng, M., Kuang, H., & Xu, C. (2015). Ultrasensitive immunochromatographic assay for the simultaneous detection of five chemicals in drinking water. *Biosensors and Bioelectronics*, 66, 445–453.
- Xu, N., Xu, L., Ma, W., Kuang, H., & Xu, C. (2015). Development and characterisation of an ultrasensitive monoclonal antibody for chloramphenicol. *Food and Agricultural Immunology*, 26(3), 440–450.
- Xu, N., Xu, L., Ma, W., Liu, L., Kuang, H., & Xu, C. (2015). An ultrasensitive immunochromatographic assay for non-pretreatment monitoring of chloramphenicol in raw milk. *Food and Agricultural Immunology*, 26(5), 635–644.
- Yao, L., Liu, L., Song, S., Kuang, H., & Xu, C. (2017). Development of indirect competitive enzyme-linked immunosorbent and immunochromatographic strip assays for carbofuran detection in fruits and vegetables. *Food and Agricultural Immunology*, 28(4), 639–651.
- Yikilmaz, Y., & Filazi, A. (2015). Detection of florfenicol residues in salmon trout via GC–MS. *Food Analytical Methods*, 8(4), 1027–1033.
- Zou, S., Cui, G., Liu, L., Song, S., & Kuang, H. (2017). Development of ic-ELISA and an immunochromatographic strip assay for the detection of methylmercury. *Food and Agricultural Immunology*, 28(4), 699–710.