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Research Article

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Gold Nanoparticle-Based Paper Sensor for Highly Specific Detection of Ofloxacin in Beef

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Abstract

Ofloxacin (OFL), a third-generation fluoroquinolone, is widely used as an antibacterial drug to prevent diseases in livestock and poultry. In this study, a paper sensor based on gold nanoparticle and a highly specific monoclonal antibody (mAb) against OFL was prepared to detect OFL residues in beef. The limit of detection of the developed immunochromatographic strip for OFL reached 0.16 ng/mL, and the linear range was from 3.125 ng/mL to 100 ng/mL, with the following optimal parameters for the preparation of colloidal gold-labeled mAb probe: pH 6.5, 2 mg/mL OFL–bovine serum albumin antigen, 10 μg/mL antibody, and 12 min immunoreaction time. Cross-reactivity (CR) experiment indicated that the developed strip is highly specific and features low CR with fluoroquinolone analogues. Overall, the developed strip is reliable for the rapid detection of OFL in beef and can be considered an effective screening method for food safety and quality management.

Keywords

Ofloxacin, Rapid detection, Monoclonal antibody, Immunochromatographic strip, Beef

Introduction

Fluoroquinolones, which are a common group of broad-spectrum synthesized antibiotics derived from quinolone nalidixic acid, are widely used in livestock to prevent diseases and promote growth [1]. Ofloxacin (OFL), which is a third-generation fluoroquinolone, is one of the most widely used potent orally absorbed antibacterial drugs [2]. The abuse of OFL, however, easily leads to a series of side effects, including phototoxicity, hemolytic–uremic syndrome, thromboembolism, and central nervous system damage [3, 4]. The long-term accumulation of OFL in human body by ingesting food with OFL residues has considerably contributed to the problem of antimicrobial resistance, which could cause the loss of potency of medicines over time [5]. Considering the above conditions, the production, management, and use of OFL in animal food products were consequently prohibited by the Ministry of Agriculture of China in 2016 [3].

Currently, the means of monitoring OFL include instrumental analytical methods, such as high-performance liquid chromatography (HPLC) [6] and liquid chromatography–mass spectrometry (LC–MS), and immunochemical methods, such as enzyme-linked immunosorbent assay (ELISA) [7-9], immunochromatographic strip [10, 11], and electrochemical optical biosensors

[12-16]. R.M.D. Byrro et al. developed and validated a sensitive liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-MS/MS) method to quantitate OFL [17]. The instrumental analytical methods are excellent corroborant means that they can simultaneously detect OFL and other veterinary drugs quantitatively and qualitatively [18]. However, the requirements of highly trained personnel, bulky apparatus, and complicated preparation limit their convenient and timely applications outdoors [19]. Immunochromatographic strip, which is one of the most widely used preliminary screening tools, became an alternative owing to its simple, rapid, and low-cost features [19]. Multiresidue immunoassays for detecting OFL have been reported over the past few decades [20-25]. X. Chen et al. reported the indirect simultaneous competitive immunoassay screening for marbofloxacin and OFL residues with half maximal inhibitory concentration (IC_{50}) of 0.76 ± 0.12 ng/mL for marbofloxacin and 2.70 ± 0.28 ng/mL for OFL [19]. Y. Wu et al. screened an anti-enrofloxacin and OFL monoclonal antibody (mAb) with IC_{50} of 6.67 and 7.13 ng/mL, respectively, and developed an immunochromatographic assay with decision limits of 0.089 and 0.217 ng/mL [26]. B.N. Tochi et al. successfully developed an anti-OFL mAb-based ELISA with IC_{50} of 1.17 ng/mL [27]. N.A. Byzova et al. developed an immunochromatographic assay to detect OFL using native antiserum New Zealand rabbits instead of purified immunoglobulins or specific antibodies labeled by colloidal gold [28].

To date, few reports exist on the detection of OFL, specifically by immunochromatographic strip based on mAb. The present study was designed to develop a highly specific immunochromatographic strip based on mAb to detect OFL residues in beef.

Materials and Methods

Reagents and instruments

Fluoroquinolone standards, including OFL, moxifloxacin (MOXI), gatifloxacin (GAT), balofloxacin (BALO), orbifloxacin (ORBI), pazufloxacin (PAZU), enrofloxacin (ENRO), and pefloxacin (PEF), were purchased from J&K Scientific Ltd (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysulfosuccinimide (NHS), Freund's incomplete adjuvant, Freund's complete adjuvant, bovine serum albumin (BSA), and ovalbumin (OVA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). A goat antimouse IgG antibody was purchased from Jackson ImmunoResearch Laboratories (PA, USA). Cell fusion reagents, such as HAT and HT solutions, were purchased from Thermo Fisher Scientific Inc. (Shanghai, China). Other reagents and chemicals were acquired from the National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China).

The sample pad, nitrocellulose (NC) membrane, and absorbent pad were purchased from Millipore, Inc. (Bedford, MA). The BioDot XYZ platform combined motion control with BioJet Quanti3050k and AirJet Quanti3050k dispensers were supplied by BioDot (Irvine, CA). A vacuum drying oven was purchased from Shanghai Fumma Laboratory Instrument Co., Ltd. (Shanghai, China). High-speed freezing centrifuge was purchased from Xiang Yi (Hunan, China). A portable test strip reader (HELMEN) was obtained from Zhejiang Fenghang Science Instrument Co., Ltd. (Zhejiang, China). The K5600 Micro-Spectrophotometer was purchased from Kaiao Co. (Beijing, China) to record the UV-via absorption spectra. The JEM-2100 Electron Microscope was purchased from JEOL Co., Ltd. (Japan).

Beef was purchased from a local supermarket. Eight-week-old female BALB/c mice were obtained from Nanchang University Laboratory Animal Center (Nanchang, China).

Buffer solutions

The basic assay buffer: phosphate-buffered saline (PBS, 0.01 M, pH 7.4), per 1 L distilled water containing 8 g NaCl, 0.2 g KCl, 0.24 g KH_2PO_4, 3.62 g Na_2HPO_4 $12H_2O$. The washing buffer (PBST): PBS containing 0.05% (v/v) Tween 20. Antibody dilution solution: PBST containing 0.1% (w/v) gelatin. The carbonate buffer solution (CBS, 0.05 M, pH = 9.6), per 1 L distilled water containing 1.59 g $NaCO_3$, 2.93 g$NaHCO_3$. The blocking buffer: CBS containing 0.2% (w/v) gelatin. Solution A: per 1 L distilled water containing 36.8 g Na_2HPO_4, 9.33 g citric acid and 180 µl of 30% H_2O_2. Solution B: 500 mL glycol containing 300 mg TMB. The substrate solution: mixing A with B at a ratio of 5:1 (v/v).

Antigen synthesis

The antigen was synthesized by OFL coupling to BSA by the active ester method. Briefly, a mixture of 1 mM OFL, 5 mM EDC, and 5 mM NHS were dissolved in 200 µL 0.01 M phosphate buffered saline (PBS, pH = 7.4) and stirred for 4 h at 25 °C. The mixtures were then added dropwise to 10 nM BSA in 0.05 M CBS. After stirring overnight, the reactants were dialyzed for 3 days in 0.01 M PBS at 4 °C to acquire the antigen OFL–BSA. The antigen was then stored at 4 °C for further experiment. Similarly, antigen OFL–OVA was prepared by substituting BSA with OVA.

Production of mAb

Eight female BABL/C mice were immunized by hypodermic injection at interval of 3 weeks [29]. Immunization was performed by 50 µg/mouse immunogen (OFL–BSA) emulsified in Freund's incomplete adjuvant, except the first immunization of 100 µg/mouse immunogen which was emulsified in Freund's complete adjuvant. One week after the fifth immunization, the mice were tail-bled, and the serum was evaluated by indirect competitive ELISA (icELISA). The mouse with the best affinity and inhibition for OFL was sacrificed to fuse. The procedures of cell fusion were described previously [30]. Briefly, three days before fusion, the selected mouse was boosted with 25 µg immunogen mixed with saline, and SP2/0 myeloma cells were cultured. On the day of fusion, the mouse was killed, and its spleen was removed to fuse with SP2/0 myeloma cells at exponential stage. The obtained hybridoma cells were cultured in HAT solution for four days and then in HT solution for three days. Subsequently, the hybridoma cells were evaluated by screening

their supernatants by icELISA, and the selected hybridoma cells were cloned thrice by limiting dilution-forming stable cell lines. The antibody was obtained by ascite production and saturated ammonium sulfate purification method described previously [31].

icELISA

The icELISA used was conventional icELISA with slight modification [32]. Briefly, 96-well microplate was coated with 100 µL/well coating antigen OFL–OVA diluted in 0.01 M CB for 2 h at 37 °C. Another 200 µL/well blocking buffer was added after washing the plate thrice with washing solution. After reacting for 2 h at 37 °C, the plates were washed again, and 50 µL standards in PBS and 50 µL mAb in antibody dilution solution were added to react for 30 min at 37 °C. After washing, 100 µL/well horseradish peroxidase-conjugated goat-antimouse IgG (diluted 1:3000 with antibody dilution buffer) was added to react for 30 min at 37 °C. After washing, 100 µL/well substrate solution was added to react for 15 min at 37 °C, and 50 µL of 2 M H_2SO_4 was added to stop the reaction. Finally, the optical density at 450 nm was read using a microplate reader.

Immunochromatographic strip based on gold nanoparticles (AuNPs)

AuNPs

AuNPs with an average diameter of 25 nm colloidal gold were synthesized by sodium citrate reduction [33]. Briefly, 1.45 mL 1% sodium citrate solution was added rapidly to 100 mL boiling and stirred 0.01% $HAuCl_4$ solution. The color of the mixture changed to red wine within approximately 1 min, and the reaction in the following 10 min was continued in a boiling state. The solution was then cooled to 25 °C and stored at 4 °C for the following experiment. The morphology of the particles (4.78 µg/mL) were characterized by transmission electron microscopy.

Preparation of colloidal gold-labeled mAb probe

The anti-OFL mAb labeled with AuNPs was prepared as described previously [33]. Briefly, 0.1 mL purified antibody diluted by ultrapure water was added dropwise to 1 mL colloidal gold solution with the pH adjusted using 0.2 M K_2CO_3. The mixture was stirred for 60 min at 25 °C, and 0.1 mL blocking solution containing 1% (w/v) polyethylene glycol 20,000 and 10% (w/v) BSA for 30 min was added dropwise to block any unreacted sites. Then, the mixture was incubated at 25 °C for 2 h and centrifuged twice at 8500 × g at 4 °C for 30 min to remove unconjugated antibodies. Finally, the AuNP-labeled anti-OFL mAb probe was resuspended in 0.1 mL 0.01 M PBS containing 0.05% Tween 20 and 1% casein. This probe was analyzed by a UV- spectrophotometer.

Preparation and procedure of immunochromatographic strip

The immunochromatographic strip was assembled as described previously [33]. Briefly, the OFL–BSA antigen and goat antimouse IgG were spotted on the NC membrane to form the test line (T line) and control line (C line) at an interval of 5 mm, respectively. After drying at 37 °C for 6 h, the sample pad, NC membrane, and absorbent pad were attached to the middle of polyvinyl chloride support plate sequentially. Finally, the plate was cut into 3.9 mm × 60 mm strips by Guillotine cutter module and stored in a desiccator. When the test was started, the OFL–BSA antigen and goat antimouse IgG (0.6 mg/mL) were sprayed onto the NC membrane to form the T and C lines and then dried at 37 °C for 12 h. Sample solution (100 µL) was added to 50 µL AuNP-labeled mAb in the wells of a microtiter plate for 5 min incubation at RT. Then, the mixtures were pipetted to the sample well of a test strip. After another 5 min, the optical signals of the T and C lines were read by a portable test strip reader.

Optimization of immunoassay strip

Optimization of pH for preparation of AuNP-labeled mAb probe

Different pH of colloidal gold solution (pH 6.0, 6.5, 7.0, 7.5, and 8.0) were adjusted with 0.2 M K_2CO_3 before preparing the AuNP-labeled mAb probe. The negative (0.01 M PBS) and positive samples (25 ng/mL OFL standard) were detected by the assembled strip. The optimal pH was determined based on the signal intensity of T line of the negative sample and competitive inhibition ratio ($1–B/B_0$), where B_0 is the ratio of signal intensity of T line to C line of the negative sample, and B is the ratio of signal intensity of T line to C line of the positive sample [34].

Optimization of antibody amount

The AuNP-labeled mAb probe was prepared with mAb solution at concentrations of 2, 4, 6, 8, 10, and 12 µg/mL in 1 mL colloidal gold solution. The negative (0.01 M PBS) and positive samples (25 ng/mL OFL standard) were then detected by the assembled strip. The optimal concentration was also determined based on the signal intensity of T line of the negative sample and the competitive inhibition ratio ($1–B/B_0$).

Optimization of OFL–BSA antigen on T line

The OFL–BSA antigen on T lines was prepared at concentrations of 1, 2, 3, 4, and 5 mg/mL. PBS (0.01 M) and OFL standard (25 ng/mL) as the negative and positive samples, respectively, were detected by the assembled strip. The optimal concentration was also determined based on the signal intensity of T line of the negative sample and competitive inhibition ratio ($1–B/B_0$).

Optimization of immunoreaction time

One minute after the mixtures of sample and mAb-labeled probe were pipetted to the sample well for a test, the experimental result was read by the strip reader every 30 s for 30 min. Three sample concentrations (0, 25, and 50 ng/mL) were detected to obtain the optimal immunoreaction time. Three immunological kinetic curves were created by using time as the X-axis and signal intensity of the T line as the Y-axis.

Establishment of standard calibration curve

A series of OFL standard (0, 1.56, 3.125, 6.25, 12.5, 25, 50, 75, 100, 150, 200, and 300 ng/mL) was prepared. Each concentration was then utilized by the optimized strip in

triplicates. The limit of detection (LOD) is defined as the mean of the negative sample minus threefold standard deviation [35].

Specificity of immunochromatographic strip

Seven analogues of fluoroquinolone drugs, namely, MOXI, GAT, BALO, ORBI, PAZU, ENRO, and PEF, at 1000 ng/mL concentration in PBS were used to evaluate the specificity of the immunochromatographic strip. Each measurement was analyzed in three replicates.

Pretreatment and recovery test

Sample extraction and detection for the strip test were performed as described earlier [36]. Briefly, beef muscle from the local supermarket was confirmed negative by Jiangxi Entry-Exit Inspection and Quarantine Bureau using LC–MS/MS. Five grams of ground beef samples were weighed into a polypropylene centrifuge tube (50 mL) for OFL detection. The beef samples were spiked with OFL standard solution at different concentrations (10, 50, and 75 ng/g). Then, the mixture was vortexed for 10 min using 5 mL of 0.2 M acetate buffer (pH = 5.6). Finally, the supernatant was used for the following analysis after centrifugation at 8000 × g at 4 °C for 10 min.

Results and Discussion

Characterization of OFL–BSA antigen

Figure 1A indicates the synthesis route of the OFL–BSA antigen. In figure 1B, the ultraviolet–visible (UV–vis) spectrum of the OFL–BSA antigen presented two peaks at 295 and 320 nm, indicating that the absorption peak of BSA at 280 nm migrated after OFL conjugated with BSA and that characteristic absorption peaks of OFL emerged at 320 nm, respectively. Therefore, OFL was successfully conjugated with BSA.

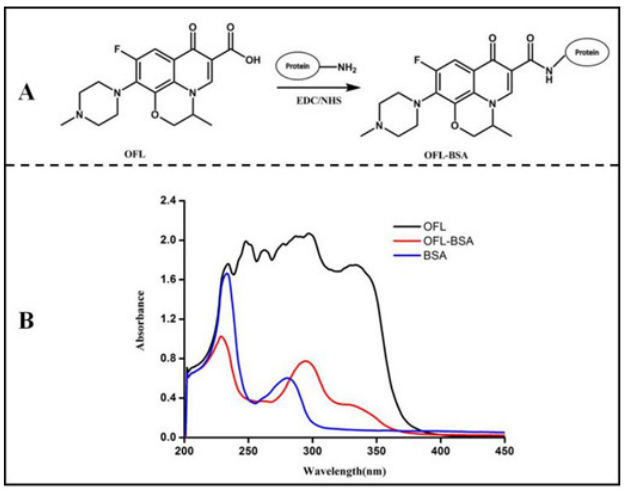

Figure 1: (A) Synthesis route of the OFL–BSA antigen. **(B)** UV–vis spectrum of the OFL, BSA and OFL–BSA antigen.

Characterization of AuNPs and AuNP-labeled mAb

As shown in figure 2, AuNPs were characterized by transmission electron microscopy (TEM) and UV–vis spectroscopy, which indicated that the prepared AuNPs with a diameter of 25 nm had excellent uniformity and dissolution. Antibodies, as biological macromolecules, could bind to the surfaces of AuNPs in the effect of high electron density. The maximum absorption wavelength for the AuNP-labeled mAb mixture was 525 nm, which indicated the formation of mAb–AuNP conjugates (Figure 2B).

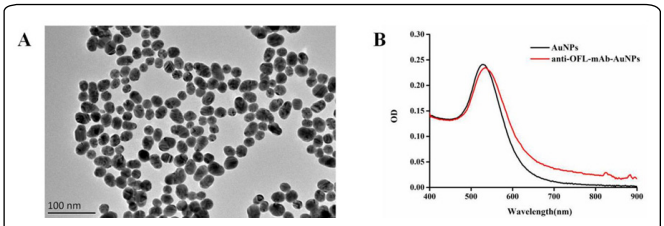

Figure 2: (A) Characterization of the AuNPs solution by TEM. **(B)** UV–vis spectrum of the AuNPs solution and AuNP-labeled mAb mixtures.

Principle of immunoassay strip

Figure 3 shows the structure and principle of the immunochromatographic strip. Owing to capillary attraction, the mixtures of sample solution and AuNP-labeled mAb flowed from the sample pad to the absorption pad, passing by the NC membrane to react with OFL–BSA antigen (T line) and goat antimouse IgG (C line). In the OFL-positive sample, a light color formed on the T line, as the AuNP-labeled mAb combined with OFL in the sample rather than with the coating antigen on the T line. The color of the T line would disappear when the concentration of OFL in the sample increased to certain amount. In the OFL-negative sample, a deep color formed owing to the AuNP-labeled mAb completely captured by the coating antigen on T line. In any case, a deep red color would form on the C line, because the AuNP-labeled mAb or the OFL–AuNP-labeled mAb mixtures would continually migrate and react with the goat–mouse IgG antibody on the C line.

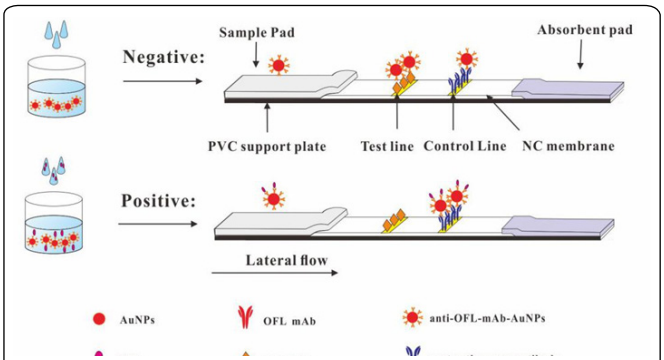

Figure 3: Structure and principle of immunochromatographic strip.

Optimization of immunoassay strip

Four parameters, namely, the pH in preparing colloidal gold-labeled mAb probe, antibody amount, OFL–BSA antigen on T lines, and immunoreaction time of immunoassay strip were optimized.

The isoelectric point (pI) of the antibody was about 8.0.

The effect of pH in preparing colloidal gold-labeled mAb probe on the results was explored. The results in figure 4A indicate that the signal intensities of T line of the negative sample reached 905, 822, 810, 476, and 410 when the pH were 6.0, 6.5, 7.0, 7.5, and 8.0 respectively in preparing colloidal gold-labeled mAb probe. As shown in figure 4A, the signal intensity of T line of the negative sample was the highest at pH 6.0 and was slightly lower when the pH was 6.5. The competitive inhibition ratio $1–B/B_0$ was the highest at pH 6.5. Therefore, we selected pH 6.5 as the optimal pH to prepare colloidal gold-labeled mAb probe. The pH in preparing colloidal gold-labeled mAb probe can affect coupling efficiency and antibody activity, it was obvious that a lower reaction pH led to a higher increase in the binding amounts of antibody [37].

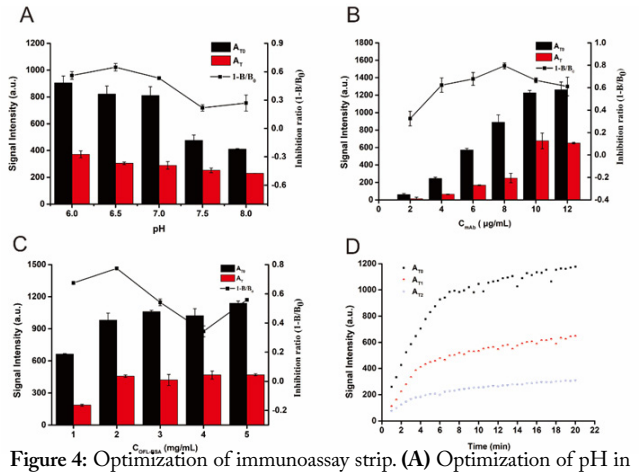

Figure 4: Optimization of immunoassay strip. **(A)** Optimization of pH in preparing colloidal gold-labeled mAb probe. Signals of T line with different concentration of OFL (0, 25 ng/mL), and the competitive inhibition ratio. **(B)** Optimization of antibody amount. Signals of T line with different concentration of OFL (0, 25 ng/mL), and the competitive inhibition ratio. **(C)** Optimization of the OFL–BSA antigen on T lines. Signals of T line with different concentration of OFL (0, 25 ng/mL), and the competitive inhibition ratio. **(D)** Optimization of immunoreaction time. Signals of T line with different concentration of OFL (0, 25, 50 ng/mL).

Figure 4B depicts that at 8 μg/mL antibody, the competitive inhibition ratio $1–B/B_0$ was the highest, whereas the signal intensity of T line of the negative sample (968) was considerably weaker than that of the 10 μg/mL sample (1198). Considering the competitive inhibition ratio $1–B/B_0$ and the signal intensity of the T line of the negative sample, we selected the antibody amount of 10 μg/mL as the optimal parameter of the test strip. When the amount of antibody increased, the more Fc terminals were toward the surface of AuNPs, and more antigen-binding fragments (Fab) were accessible for antigens [37], thus the signal intensity of the T lines increased, but at the same time the goat–mouse IgG antibody on the C line could capture more AuNP-labeled mAb or the OFL–AuNP-labeled mAb mixtures to show a strong signal intensity, so competitive inhibition ratio would be affected and should be considered.

The signal intensity of the T line increased when the concentration of OFL–BSA increased from 1mg/mL to 5 mg/mL. As the concentration increasing from 1 mg/mL to 2 mg/mL, the T-line signal intensity was significantly enhanced, then the signal intensity grew slowly (Figure 4C). It would be that antigen on the T-line was nearly saturated for the AuNP-labeled mAb. The maximum competitive inhibition ratio was observed when the concentration of OFL–BSA antigen was 2.0 mg/mL. Therefore, 2.0 mg/mL was regarded as the optimal concentration of OFL–BSA on the T line.

As shown in figure 4D, three immunological kinetic curves were created. A_{T0}, A_{T1}, and A_{T2} represent the signal intensities of T line on the negative sample (0 ng/ml), 25 ng/ml positive sample, and 50 ng/ml positive sample, respectively. The signal intensity of T line increased with time and stabilized after 12 min. The AuNP-labeled mAb or the OFL–AuNP-labeled mAb mixtures must have migrated and reacted completely with coating antigen (T line) and goat–mouse IgG antibody (C line). Therefore, 12 min was selected as the optimal immunoreaction time.

Immunochromatographic strip assay

The test strip was evaluated in PBS and beef samples. The beef sample was confirmed negative by LC/MS/MS. OFL standard was dissolved in 0.05 M Na_2CO_3 to obtain a stock solution at 1 mg/mL concentration. Under the optimized conditions, a series of concentrations of OFL standards (0, 1.56, 3.125, 6.25, 12.5,25, 50, 75, 100, 150, 200, and 300 ng/mL) was prepared by diluting with 0.01 M PBS and evaluated by the test strip with three replicates. As shown in figure 5, a calibration curve was constructed by plotting $1–B/B_0$ against the logarithm of a series of concentrations of OFL standards. The regression equation is represented by y = 0.3165x + 0.2798, where y is the competitive inhibition ratio $1–B/B_0$, and x is the concentration of OFL standards, with a good linear (from 3.125 ng/mL to 100 ng/mL) and a reliable correlation coefficient (R^2 = 0.9891). The LOD of the strip was 0.16 ng/mL by plugging the values of negative sample minus threefold standard deviation into the equation.

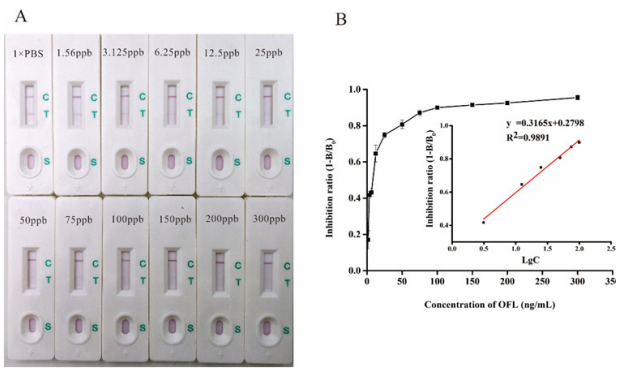

Figure 5: (A) Images of OFL detection using the immunochromatographic strip. **(B)** Standard calibration curve for OFL.

Specificities of the test strip

In this work, 0.01 M PBS, 100 ng/mL OFL, and seven fluoroquinolone drugs, namely, MOXI, GAT, BALO, ORBI, PAZU, ENRO, and PEF, at 100 ng/mL concentration in PBS were evaluated by the developed strip. Figure 6 shows the distinct color of the T line on fluoroquinolone drug strips, the T line color of 100 ng/mL OFL strip completely

disappeared, and except for structurally related analogs, PAZU, the developed assay possessed high specificity with others six fluoroquinolone drugs.

Figure 6: Specificities of the test strip. From left to right: 0.01 M PBS, OFL (100 ng/mL), MOXI (100 ng/mL), GAT (100 ng/mL), BALO (100 ng/mL), ORBI (100 ng/mL), PAZU (100 ng/mL), ENRO (100 ng/mL), and PEF (100 ng/mL).

Recovery experiment for OFL in meat

Recovery experiments for OFL were performed using the developed strip in beef samples. As shown in table 1, the samples with same spiked concentration were analyzed in three replicates. The recoveries ranged from 57.13% to 104.42%, the standard deviation (SD) ranged from 0.78 to 8, and the coefficient of variation (CV) ranged from 4.08 %~15.93 %, which could prove good stability and reproducibility of this method. When the spiked concentration was 10 ng/mL, the recovery rate was low. It could be guessed that the lower the spiked concentration, the more significant the effect of the matrix. In general, the developed strip is reliable for determining OFL in beef.

Table 1: Recoveries of OFL in beef by the developed strip.

Spiked OFL (ng/g)	Mean (ng/g)	SD	Recovery[1] (%)	CV[2](%)
10	5.71	0.78	57.13	13.66
50	52.21	8.32	104.42	15.93
75	59.62	2.43	79.49	4.08

[1]Recovery = (detection concentration / spiked concentration) × 100%

[2]CV = (SD / mean) × 100%

Conclusion

In this study, an immunochromatographic strip based on a highly specific mAb against OFL was prepared for OFL detection in beef. Under optimized parameters of pH 6.5, 2 mg/mL OFL–BSA antigen, 10 μg/mL antibody, and 12 min immunoreaction time for the preparation of colloidal gold-labeled mAb probe, the LOD of the developed strip for OFL reached 0.16 ng/mL, and the linear range was from 3.125 ng/mL to 100 ng/mL. CR experiment indicated high specificity with six fluoroquinolone drugs namely, MOXI, GAT, BALO, ORBI, ENRO, and PEF, except for PACU. False positive results can decrease, given that the strip can detect OFL specifically. The developed strip was shown to be rapid and reliable for detecting OFL in beef and can be considered an effective screening method for food safety and quality management.

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References

1. Fernandez F, Pinacho DG, Gratacos-Cubarsi M, Garcia-Regueiro JA, et al. 2014. Immunochemical determination of fluoroquinolone antibiotics in cattle hair: a strategy to ensure food safety. *Food Chem* 157: 221-228. https://doi.org/10.1016/j.foodchem.2014.01.119
2. Tochi BN, Peng J, Song S, Liu L, Kuang H, et al. 2016. Determination of sarafloxacin and its analogues in milk using an enzyme-linked immunosorbent assay based on a monoclonal antibody. *Anal Methods* 8(7): 1626-1636. https://doi.org/10.1039/C5AY02702K
3. Peng J, Liu L, Kuang H, Cui G, Xu C. 2016. Development of an icELISA and immunochromatographic strip for detection of norfloxacin and its analogs in milk. *Food Agric Immunol* 28(2): 288-298. https://doi.org/10.1080/09540105.2016.1263987
4. Yan ZY, Yi HY, Wang LM, Zhou XT, Yan R, et al. 2019. Fluorescent aptasensor for ofloxacin detection based on the aggregation of gold nanoparticles and its effect on quenching the fluorescence of Rhodamine B. *Spectrochim Acta A Mol Biomol Spectrosc* 221: 117203. https://doi.org/10.1016/j.saa.2019.117203
5. Peng J, Liu L, Xu L, Song S, Kuang H, et al. 2016. Gold nanoparticle-based paper sensor for ultrasensitive and multiple detection of 32 (fluoro)quinolones by one monoclonal antibody. *Nano Res* 10(1): 108-120. https://doi.org/10.1007/s12274-016-1270-z
6. Shah P, Pandya T, Gohel M, Thakkar V. 2018. Development and Validation of HPLC method for simultaneous estimation of Rifampicin and Ofloxacin using experimental design. *J Taibah Univ* Sci 13(1): 146-154. https://doi.org/10.1080/16583655.2018.1548748
7. Hou XL, Guo KJ, Bao J, Wu YN, Li HR. 2011. Development of an enzyme-linked immunosorbent assay for detection of ofloxacin residue in environment matrix. *J Food Agric Environ* 9(3-4): 779-783. https://doi.org/10.1234/4.2011.2410
8. Shanin IA, Thuy NTD, Eremin SA. 2014. Determination of levofloxacin (the Levorotatory Stereoisomer of Ofloxacin) in milk by indirect enzyme-linked immunosorbent assay. Moscow Univ Chem Bull 69: 136-141. https://doi.org/10.3103/S0027131414030080
9. Zhang L, Jiang JQ, Li GL, Wang ZL, Yang XF, et al. 2011. Development and optimization of a monoclonal antibody-based indirect competitive ELISA for detecting fluoroquinolone residue in milk. *J Food Agric Environ* 9(2): 113-120. https://doi.org/10.1234/4.2011.2071
10. Sun WY, Liu WY, Qu LB. 2007. Development of ELISA and immunochromatographic assay for ofloxacin. *Chin Chem Lett* 18(9): 1107-1110. https://doi.org/10.1016/j.cclet.2007.07.008
11. Zhu Y, Li L, Wang ZH, Chen YQ, Zhao ZM, et al. 2008. Development of an immunochromatography strip for the rapid detection of 12 fluoroquinolones in chicken muscle and liver. *J Agric Food Chem* 56(14): 5469-5474. https://doi.org/10.1021/jf800274f
12. Hu S, Li DH, Huang Z, Xing KY, Chen Y, et al. 2018. Ultra-sensitive method based on time-resolved fluorescence immunoassay for detection of sulfamethazine in raw milk. *Journal of Food and Agricultural Immunology* 29(1): 1137-1149. https://doi.org/10.1080/09540105.2018.1520816
13. Shi QQ, Huang J, Sun YN, Deng RG, Teng M, et al. 2018. A SERS-based multiple immuno-nanoprobe for ultrasensitive detection of neomycin and quinolone antibiotics via a lateral flow assay. *Microchimica Acta* 185: 84. https://doi.org/10.1007/s00604-017-2556-x

14. Wong A, Silva TA, Vicentini FC, Fatibello O. 2016. Electrochemical sensor based on graphene oxide and ionic liquid for ofloxacin determination at nanomolar levels. *Talanta* 161: 333-341. https://doi.org/10.1016/j.talanta.2016.08.035
15. Taranova NA, Berlina AN, Zherdev AV, Dzantiev BB. 2015. 'Traffic light' immunochromatographic test based on multicolor quantum dots for the simultaneous detection of several antibiotics in milk. *Biosens Bioelectron* 63: 255-261. https://doi.org/10.1016/j.bios.2014.07.049
16. Li J, Zhao K, Hong X, Yuan H, Ma L, et al. 2005. Prototype of immunochromatographic assay strips using colloidal CdTe nanocrystals as biological luminescent label. *Colloids Surf B Biointerfaces* 40(3-4): 179-182. https://doi.org/10.1016/j.colsurfb.2004.10.017
17. Byrro RMD, Fulgencio GD, Cunha AD, Cesar IC, Chellini PR, et al. 2012. Determination of ofloxacin in tear by HPLC-ESI-MS/MS method: comparison of ophthalmic drug release between a new mucoadhesive chitosan films and a conventional eye drop formulation in rabbit model. *J Pharm Biomed Anal* 70: 544-548. https://doi.org/10.1016/j.jpba.2012.05.003
18. Hu K, Huang X, Jiang Y, Fang W, Yang X. 2010. Monoclonal antibody based enzyme-linked immunosorbent assay for the specific detection of ciprofloxacin and enrofloxacin residues in fishery products. *Aquaculture* 310(1-2): 8-12. https://doi.org/10.1016/j.aquaculture.2010.08.008
19. Chen X, Li Z, Guo J, Li D, Gao H, et al. 2017. Simultaneous screening for marbofloxacin and ofloxacin residues in animal-derived foods using an indirect competitive immunoassay. *Journal of Food and Agricultural Immunology* 28(3): 489-499. https://doi.org/10.1080/09540105.2017.1297780
20. Li YL, Ji BQ, Chen W, Liu LQ, Xu CL, et al. 2008. Production of new class-specific polyclonal antibody for determination of fluoroquinolones antibiotics by indirect competitive ELISA. *Journal of Food and Agricultural Immunology* 19(4): 251-264. https://doi.org/10.1080/09540100802471538
21. Liu YZ, Zhao GX, Liu J, Zhang HC, Wang P, et al. 2013. Synthesis of novel haptens against ciprofloxacin and production of generic monoclonal antibodies for immunoscreening of fluoroquinolones in meat. *J Sci Food Agric* 93(6): 1370-1377. https://doi.org/10.1002/jsfa.5901
22. Liu YZ, Zhao GX, Wang P, Liu J, Zhang HC, et al. 2013. Production of the broad specific monoclonal antibody against sarafloxacin for rapid immunoscreening of 12 fluoroquinolones in meat. *J Environ Sci Health B* 48(2): 139-146. https://doi.org/10.1080/03601234.2013.727668
23. Pinacho DG, Sanchez-Baeza F, Marco MP. 2012. Molecular modeling assisted hapten design to produce broad selectivity antibodies for fluoroquinolone antibiotics. *Anal Chem* 84(10): 4527-4534. https://doi.org/10.1021/ac300263m
24. Sheng W, Li Y, Xu X, Yuan M, Wang S. 2011. Enzyme-linked immunosorbent assay and colloidal gold-based immunochromatographic assay for several (fluoro)quinolones in milk. *Microchimica Acta* 173(3-4): 307-316. https://doi.org/10.1007/s00604-011-0560-0
25. Wang WS, Shih CW, Hung SW, Ling YF, Tu CY, et al. 2006. Development and application of the enzyme-linked immunosorbent assay residual detection kits for fluoroquinolones. *Taiwan Veterinary Journal* 32(4): 301-311.
26. Wu YC, Guo S, Dong Q, Song YZ. 2016. Development of an immunochromatographic test strip for rapid simultaneous detection of enrofloxacin and ofloxacin in tissue of chicken muscle and pork. *Food Anal Methods* 9(10): 2807-2813. https://doi.org/10.1007/s1216
27. Tochi BN, Peng J, Song S, Liu L, Kuang H, et al. 2016. Production and application of a monoclonal antibody (mAb) against ofloxacin in milk, chicken and pork. *Journal of Food and Agricultural Immunology* 27(5): 643-656. https://doi.org/10.1080/09540105.2016.1148125
28. Byzova NA, Smirnova NI, Zherdev AV, Eremin SA, Shanin IA, et al. 2014. Rapid immunochromatographic assay for ofloxacin in animal original foodstuffs using native antisera labeled by colloidal gold. *Talanta* 119: 125-132. https://doi.org/10.1016/j.talanta.2013.10.054
29. Zhao L, Li J, Li Y, Wang T, Jin X, et al. 2017. Preparation of monoclonal antibody and development of an indirect competitive enzyme-linked immunosorbent assay for ornidazole detection. *Food Chem* 229: 439-444. https://doi.org/10.1016/j.foodchem.2017.02.100
30. Song YH, Song SS, Liu LQ, Kuang H, Guo LL, et al. 2016. Simultaneous detection of tylosin and tilmicosin in honey using a novel immunoassay and immunochromatographic strip based on an innovative hapten. *Journal of Food and Agricultural Immunology* 27(3): 314-328. https://doi.org/10.1080/09540105.2015.1089843
31. Kuang H, Xing CR, Hao CL, Liu LQ, Wang LB, et al. 2013. Rapid and highly sensitive detection of lead ions in drinking water based on a strip immunosensor. *Sensors (Basel)* 13(4): 4214-4224. https://doi.org/10.3390/s130404214
32. Sheng W, Xia XF, Wei KY, Li J, Li QX, et al. 2009. Determination of marbofloxacin residues in beef and pork with an enzyme-linked immunosorbent assay. *J Agric Food Chem* 57(13): 5971-5975. https://doi.org/10.1021/jf900940n
33. Wu CH, Liu DF, Peng T, Shan S, Zhang GG, et al. 2016. Development of a one-step immunochromatographic assay with two cutoff values of aflatoxin M1. *Food Control* 63: 11-14. https://doi.org/10.1016/j.foodcont.2015.11.010
34. Hu LM, Luo K, Xia J, Xu GM, Wu CH, et al. 2017. Advantages of time-resolved fluorescent nanobeads compared with fluorescent submicrospheres, quantum dots, and colloidal gold as label in lateral flow assays for detection of ractopamine. *Biosens Bioelectron* 91: 95-103. https://doi.org/10.1016/j.bios.2016.12.030
35. Wang C, Peng J, Liu DF, Xing KY, Zhang GG, et al. 2018. Lateral flow immunoassay integrated with competitive and sandwich models for the detection of aflatoxin M_1 and *Escherichia coli* O157:H7 in milk. *J Dairy Sci* 101(10): 8767-8777. https://doi.org/10.3168/jds.2018-14655
36. Liu LQ, Peng J, Xie ZJ, Song SS, Kuang H, et al. 2017. Development of an icELISA and immunochromatographic assay for Methyl-3-Quinoxaline-2-Carboxylic acid residues in fish. *Food Anal Methods* 10(9): 3128-3136. https://doi.org/10.1007/s12161-017-0888-0
37. Lou DD, Ji L, Fan L, Ji YX, Gu N, et al. 2019. Antibody-oriented strategy and mechanism for the preparation of fluorescent nanoprobes for fast and sensitive immunodetection. *Langmuir* 35(14): 4860–4867. https://doi.org/10.1021/acs.langmuir.9b00150