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Preparation of a highly specific and sensitive monoclonal antibody against tilmicosin and its application in lateral flow immunoassay

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Abstract

To reduce the false positive results caused by cross reactivity of the antibodies with other structural analogues, it is crucial to prepare a high specificity and sensitivity antibody against target for developing an accurate immunoassay. In this study, tilmicosin (TM) was selected as a model molecule. Firstly, two-dimensional similarity, electrostatic potential energy, mulliken atomic charges and overlapping of different haptens with TM were calculated using Gaussian 09W and Discovery studio, and the newly designed TM-HS was selected as the optimal hapten. Furthermore, a monoclonal antibody (mAb 12C8) was produced with the half maximal inhibitory concentration (IC_{50}) of 0.36 ng/mL, and negligible cross-reactivity (CR) with other antibiotics. Finally, a lateral flow immunoassay (LFA) for the detection of TM based on amorphous carbon nanoparticles (ACNPs) labeled mAb 12C8 was developed by the reflectance value under natural light. The recoveries of TM ranged from 83.18% to 103.25% with a coefficient of variation (CV) < 12.47%. The results showed that the cut-off value of TM in milk samples was 1 ng/mL, and the limits of detection (LODs) for chicken muscle, bovine muscle, porcine muscle and porcine liver samples were 5.23, 5.98, 6.85 and 7.31 μg/kg, respectively. In addition, 40 real samples were tested by the LFA, and the detection results were consisted with that of high-performance liquid chromatography-UV detector (HPLC–UV). Those results indicated that the developed LFA is an accurate and useful tool for on-site screening of TM in milk and animal tissues.

Keywords Hapten design, Monoclonal antibody, High sensitivity, Tilmicosin, Lateral flow immunoassay

Introduction

Tilmicosin (TM) is a macrolide antibiotic with a 16-carbon lactone ring, mainly used for the treatment of aerobic gram-positive cocci and gram-negative cocci [1, 2], and widely used to treat bacterial infections in a variety of animals, including beef cattle, swine and poultry [3, 4]. In addition, as a feed additive, it could promote animal growth and improve feed utilization [5]. However, overuse of TM may lead to its residue in the edible tissue of animals that result in allergic reactions and bacterial drug resistance [6, 7]. To protect human health from TM residues, the maximum residue limits (MRLs) for TM in milk, beef, pork, chicken and pig liver were set at 50, 100, 100, 150, and 1500 μg/kg, respectively [8]. Therefore, it is urgent to establish a rapid

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analytical method for the accurate detection of TM in milk samples and edible animal tissues.

Nowadays, several analytical methods have been reported for the detection of TM, such as high-performance liquid chromatography-UV detector (HPLC-UV), high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), and immunoassays [9–12]. Although instrumental analysis methods are sensitive, and reliable, they are unsuitable for on-site screening due to time-consuming nature. Enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFA) are two common immunoassays [13, 14]. Among them, LFA is extensively preferred over ELISA in on-site screening due to its speed, simplicity, operability and portability. High sensitivity and specificity of the antibody is a prerequisite for the development of an accurate immunoassay. According to the performance of reported anti-TM antibodies (Table 1), these antibodies could recognize tylosin (TYL, a structural analogues of TM), or exhibit low sensitivity (the half maximal inhibitory concentration (IC_{50}) > 1.4 ng/mL) [15–18]. Low-specificity antibodies may cause false-positive results, and low-sensitivity antibodies may fail to detect trace targets, limiting the application of this antibody. Thus, a new specific antibody with high specificity and sensitivity against TM is necessary for developing an accurate immunoassay.

The similarity between the haptens and the target, such as two-dimensional (2D) similarity, electrostatic potential energy (ESP), spatial conformation, and charge distribution are the main factors affecting the preparation of highly specific and sensitive antibodies [19]. We previously prepared a specific antibody by designing a novel hapten 4-(((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4yl)amino)methyl) benzoic acid, which possessed structural features almost identical to the target of 4-methylaminoantipyrine, for its detection. The IC_{50} of the produced monoclonal antibody (mAb) 6A4 was 4.03 ng/mL, with negligible cross-reactivity (CR) to other dipyrone metabolites [20]. In addition, a new hapten 6-(aminomethyl)-3- methylquinoxaline-2-carboxylic acid, maintaining all structural features of 3-methyl-quinoxaline-2-carboxylic acid was prepared. The resulting mAb exhibited an IC_{50} value of 0.2 μg/L, yielding a 15.5- to 88.5-fold improvement compared to previously prepared specific antibodies against 3-methyl-quinoxaline-2-carboxylic acid [21]. The above results demonstrate that the designed hapten should retain the common structure of the target compound to ensure the highest similarity between the hapten and the target [22]. Thus, analyzing the similarity between the reported haptens and TM would further help in obtaining the optimal hapten, producing a highly specificity and sensitivity antibody against TM for the development of an accurate immunoassay.

Table 1 2D similarities of TM with haptens

	TM-H1	TM-H2	TM-HS
TM	94.3%	96.5%	97.5%

LFAs have emerged as universal techniques for on-site screening of TM. Colloidal gold nanoparticles (CGNPs) mainly used as tracers in LFAs because qualitative results can be rapidly obtained, but the sensitivity is poor due to its low molar absorbance coefficient [23]. To establish highly sensitive LFAs, novel nanomaterials such as amorphous carbon nanoparticles (ACNPs) that can obtain signals by the naked eye should be explored. ACNPs are more sensitive than CGNPs when utilized as labels in LFAs due to their strong dark color and high contrast against light backgrounds [24]. To our knowledge, there are no studies evaluating the feasibility of ACNPs-based LFAs for TM detection.

In this study, an accurate LFA was developed based on a high specificity and sensitivity antibody labelled ACNPs as probes. Firstly, we determined the 2D similarity, ESP, spatial conformation and charge distribution of haptens TM-H1, TM-H2, TM-HS, and TM using Discovery Studio 2019, Gaussian 09W and GaussView 5.0 software. Subsequently, we identified the optimal hapten based on its highest molecular similarity to TM, and then the hapten was conjugated with a carrier protein to prepare the mAb with high specificity and sensitivity. Finally, an accurate LFA based on new antibody labelled ACNPs was developed for the detection of TM in milk and edible animal tissues.

Results and discussion

Hapten design

Antibodies with high specificity and sensitivity are key factors affecting the accuracy of immunoassays. In antibody preparation, the design of the hapten can directly affects the performance of the antibodies. Some antibodies against TM have been reported in Table 2, which are mainly divided into two categories. One is broad-spectrum antibodies, which recognize tylosin [15, 16] and may lead to false positive results. The other is specific antibodies, which can only recognize TM, however, these antibodies have low sensitivity [17, 18]. Therefore, preparing a high specificity and sensitivity antibody against TM is necessary to establish an accurate immunoassay with highly sensitivity.

According to the references, two pathways for hapten design were explored to generate antibodies against TM (Table 2). First, the active amidogen of the derivative of TM from C13 was introduced on the spacer arm, resulting in TM-H1 [17]. The other way is to insert a

Table 2 Performance comparison of reported TM antibodies

Hapten	Immunogen hapten	Antibody	IC_{50} (ng/mL)	CR with TYL
TYL-CHO	TYL-CMO-BSA	mAb [15]	9.6	204.25%
TYL-CMO	TYL-CMO-BSA	mAb 2B3 [16]	2.1	368.42%
TM-H1	OMT-H1-KLH	mAb Til-1 [17]	48	-
		mAb Til-2 [17]	43.5	-
		mAb Til-3 [17]	54	-
		mAb Til-4 [17]	37.5	-
		mAb Til-5 [17]	32	-
		mAb Til-6 [17]	38	-
TM-H2	TM-H1-BSA	mAb [18]	1.40	-
TM-HS	TM-HS-KLH	mAb 12C8 [this work]	0.36	-

"-" means no CR with TYL

2- (4-bromobutyl) -1, 3-dioxopentylene group from N56 of TM to prepare TM-H2 [18]. According to the chemical construction of TM, the hydroxyl of TM could react with HS, named TM-HS. In addition, the 2D similarity, ESP, Mulliken atomic charges and overlapping between the haptens (TM-H1, TM-H2 and TM-HS) and TM were calculated. The 2D similarity of those haptens TM-HS with TM were 94.3%, 96.5% and 97.5%, respectively, indicating that TM-HS was highest similarity with TM (Table 1). TM-HS had similar ESP to TM with more ESP negative regions (red) on tylonolide (Fig. 1A); the Mulliken atomic charges of TM-HS were almost the same as TM (Fig. 1B). In addition, TM-HS could overlap very well with TM at mycinose and tylonolide (Fig. 1C). Based on those results, TM-HS was considered be the best hapten for producing a high specificity and sensitivity antibody against TM.

Characterisation of complete antigens

The complete antigens were characterized using mass spectra and UV-visible absorption spectra. In Fig. 2, the mass spectrometry data displayed an m/z ratio of M 969.5468 for TM-HS, with a found [M + H] value of 970.5531, confirming the successful preparation of the TM-HS hapten. In Figure S1, TM-HS exhibited a maximum absorption peak at 289 nm, while KLH and BSA showed maximum absorption peaks were at 279.5 nm and 278.5 nm, respectively. The maximum absorbance peaks of TM-HS-KLH/BSA were observed at 283.5 nm and 280.5 nm. This shift in the maximum absorbance peak indicates a successful synthesis of the complete antigens.

Preparation of mAbs

After the third immunization, the antibody titer and inhibition rates (IR) were characterized by iELISA and indirect ELISA (icELISA) (Table S1). The concentration of the coating antigens of TM-HS-BSA was 0.2 μg/mL, and serum was diluted 8000-fold by 10 mM phosphate buffered saline (PBS). As shown in Table S1, the OD_{450} values were all > 2.03, indicating that all mice exhibited a significant immune response. From the IR results, most antisera could recognize TM, and mouse number 4 was selected for cell fusion because the IR value with TM was 57.47%.

After cell fusion, mAb 12C8 was obtained. The sensitivity of the mAb was evaluated using icELISA. As shown in Table 1, the IC_{50} value was 0.36 ng/mL, indicating that mAb 12C8 exhibited high sensitivity antibody against TM. Therefore, TM-HS-BSA and mAb 12C8 were utilized for LFA development.

Development of the LFA

The concentration of coating antigen, the usage of mAb conjugated with ACNPs, and the dosage of ACNPs-mAb can affect LFA sensitivity, hence these conditions were optimized. The colorimetric values of the T-line measured by the immunochromatographic reader were more than 800 with 0 ng/mL TM as the evaluation standard. Under these conditions, IR was selected as the evaluation criterion at 0.5 and 1 ng/mL for ACNPs-mAb of TM. Higher IR values indicated greater sensitivity of the LFA. In summary, the optimal conditions for the developed LFA were found to be 0.3 mg/mL TM-HS-BSA (Fig. 3A), 10 μg mAb 12C8 conjugated with 1 mL of ACNPs (Fig. 3B), and 3 μL of ACNPs-mAb 12C8 (Fig. 3C). The same optimization procedure was performed for CGNPs-LFA (Fig. 3D–F).

Under optimal conditions, the concentrations of TM were set at 0, 0.4, 0.6, 0.8, 1.0 and 1.2 ng/mL for ACNPs-LFA in PBST or milk samples. As shown in Fig. 3G, the cut-off value for TM was 1 ng/mL. In the semi-quantitative assay (Fig. 3H), IC_{50} value of TM was 0.52 ng/mL, and $IC_{20} \sim IC_{80}$ ranged from 0.27 to 0.87 ng/mL.

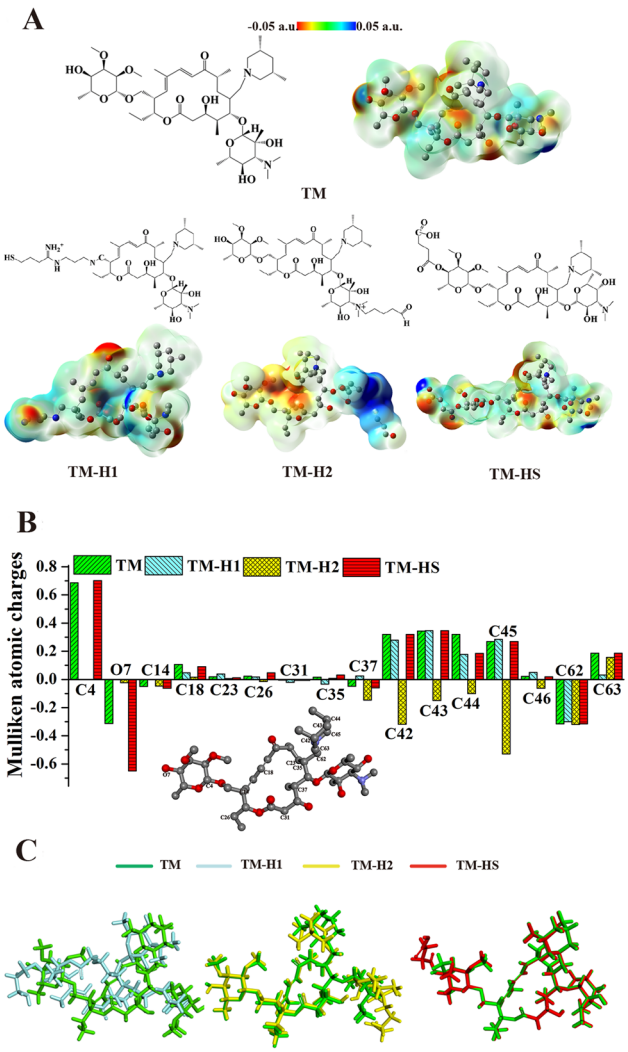

Fig. 1 ESP energy, Mulliken atomic charges and molecular overlap of haptens and target. **A** ESP energy of haptens and target. Red, blue, and white indicate negative ESP regions, positive regions, and neutral regions, separately. **B** Mulliken atomic charges of targets and haptens. Only heavy atoms belonging to targets and haptens are shown. **C** Molecular overlay based on the lowest energy conformation of TM (green), TM-H1 (blue), TM-H2 (yellow) and TM-HS (red)

For CGNPs-LFA, the concentrations of TM were set at 0, 0.4, 0.8, 1.2, 1.6 and 2.0 ng/mL in PBST or milk samples. As shown in Fig. 3G, the cut-off value for TM was 2 ng/mL. In the semi-quantitative assay, IC_{50} value of TM was 1.10 ng/mL, and IC_{20} ~ IC_{80} ranged from 0.68 to 1.78 ng/mL (Fig. 3H). The sensitivity of the ACNPs-LFA was found to be two-fold higher compared to CGNPs-LFA. In addition, the developed ACNPs-LFA had the superior sensitivity compared to previously reported LFA methods (Table 3) [15, 23–25].

To evaluate the specificity of the LFA, other structural analogues, such as TYL, tiamulin, avermectin, streptomycin, erythromycin and spiramycin, were assessed in milk at 100 ng/mL. Negligible CR with other structural analogues was observed (Fig. 3I), indicating the LFA possessed excellent specificity for TM detection.

Application of LFIA in edible animal tissue samples

The recoveries for chicken muscle, bovine muscle, porcine muscle and porcine liver of ACNPs-LFA were subsequently determined at three spiked levels (15, 20 and 25 μg/kg). The recoveries of TM ranged from 83.18% to 103.25% with CV below 12.47% (Fig. 4 and Table S2), and the LOD values were 5.23, 5.98, 6.85 and 7.31 μg/kg for chicken muscle, bovine muscle, porcine muscle and porcine liver, respectively. In addition, the accuracy of this LFA was evaluated by using 40 real samples using the LFA and high-performance liquid chromatography-UV detector (HPLC-UV). As shown in Fig. 4 and Figure S2, the concentrations in NO.3 of chicken muscle and NO.9 of porcine muscle were 62.7 and 67.2 μg/kg based on HPLC-UV, and were 55.8 and 58.4 μg/kg using the developed LFA, respectively. Those results indicate that the developed LFA was capable of accurate detection of TM in edible animal tissue samples.

Conclusions

In this study, TM-HS was utilized as the best hapten for the preparation of high specific and sensitive antibodies against TM in a 2D manner, which included ESP, Mulliken atomic charges and overlapping results. With this hapten, we developed a new mAb 12C8 against TM with IC_{50} of 0.36 ng/mL. Furthermore, we successfully devised an accurate ACNPs-LFA for TM detection in milk and edible animal tissue, demonstrating consistent results with HPLC-UV when applied to real samples. The major limitation of ACNPs-LFA is that the technology is unable to obtained the concentration of TM when detection high concentration positive sample. These results indicated that the developed ACNPs-LFA could be used as an accurate and practical tool for on-site screening of TM in milk and edible animal tissue samples.

Materials and methods

Reagents and materials

TM, tylosin, avermectin, ivermectin, streptomycin, erythromycin and ampicillin and other chemical standards were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). N, N-dimethylformamide (DMF), N, N'-dicyclohexylcarbodiimide (DCC), Tween-20, succinic anhydride, triethylamine, 4-dimethylaminopyridine (DMAP), tetramethylbenzidine (TMB), ethanol, ammonium acetate, acetonitrile and all other rest reagents

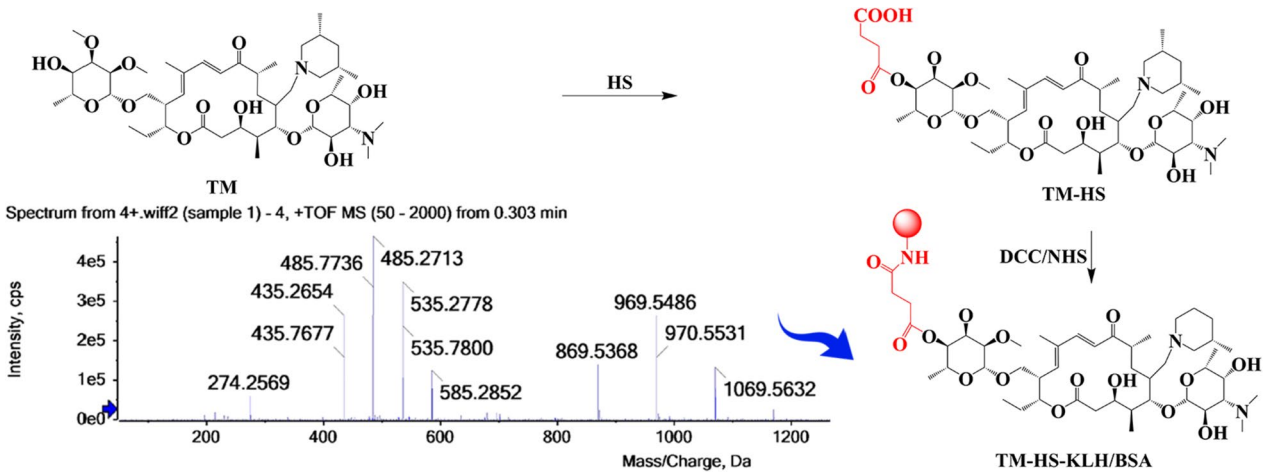

Fig. 2 The synthesis pathway and mass spectrogram of hapten of TM-HS

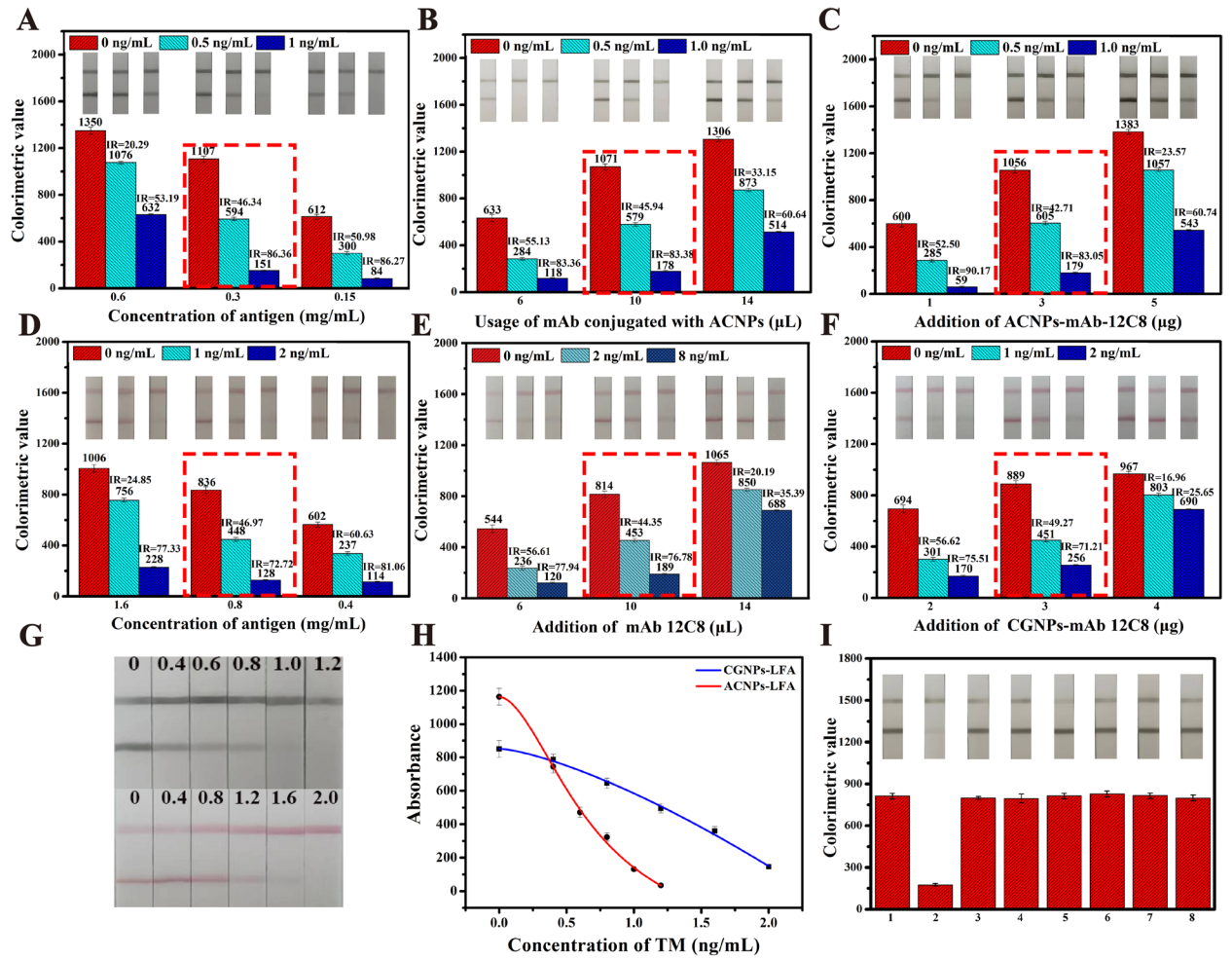

Fig. 3 LFA development. Optimization of **A** antigen concentration, **B** mAb 12C8 usage, **C** volume of ACNPs-mAb 12C8 of ACNPs-LFA, respectively. Optimization of **D** antigen concentration, **E** mAb 12C8 usage, **F** volume of CGNPs-mAb 12C8 of CGNPs-LFA, respectively. **G** Images of developed LFA. **H** Calibration curves of ACNPs-LFA and CGNPs-LFA **I** Specificity of ACNPs-LFA. 1 = PBST; 2 = TM at 1 ng/mL; 3, 4, 5, 6, 7 and 8 = tylosin, tiamulin, avermectin, streptomycin, erythromycin and spiramycin at 100 ng/mL, respectively

Table 3 Comparison of the other reported immune methods for determination of TYL and TM

Labels	Performance	CR for TYL	References
CGNPs	5 ng/mL (cut off)	300%	[15]
CGNPs	4.1 ng/mL (IC_{50})	141.37%	[24]
Fluorescent microspheres	1.01 ng/mL (IC_{50})	72.66%	[25]
CGNPs	15 ng/mL (cut off)	200%	[23]
Time-resolved fluorescent microsphere	8 ng/mL (cut off)		
TRFMs	4 ng/mL (cut off)		
CGNPs	1.10 ng/mL (IC_{50})	-	This work
ACNPs	0.52 ng/mL	-	This work

"-" Means no CR with TYL

were obtained from the National Pharmaceutical Group Chemical Reagent Co. Ltd. (Shanghai, China). All the above reagents were of analytical grade. Keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), complete Freund's adjuvants, incomplete Freund's adjuvants and skim milk were obtained from Sigma-Aldrich (St. Louis, MO, USA). Horseradish peroxidase (HRP)-labeled goat was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). Milk, chicken muscle, porcine muscle, bovine muscle and porcine liver were purchased from local supermarket. Amorphous carbon nanoparticles (ACNPs) were obtained from Beijing Najing Biological Technology Co., Ltd. (Beijing, China). 96-well polystyrene ELISA plates and Cell culture plates were supplied from Costar Corp (Cambridge, MA, USA).

The sample pad (HG-2) and PVC flooring were supplied by Shanghai Jinbiao Biotechnology Co., Ltd (Shanghai, China). The nitrocellulose (NC) filter membrane and absorption pads were obtained from Shantou Yineng Membrane Industry Co., Ltd. (Guangdong, China). The gold spraying scribing instrument (HS510) was purchased from Hangzhou Fenghang Technology Co., Ltd. (Hangzhou, China). The immunochromatographic reader was provided by Feike Instrument Co., Ltd. (Shanghai, China). Test strip slitter (ZQ2002) was purchased from Jinbiao Biotechnology Co., Ltd (Shanghai, China).

Softwares

The lowest energy conformation of molecules were optimized using Gaussian 09W software (Gaussian, Wallingford, CT, USA). The Gaussian out file provided Mulliken

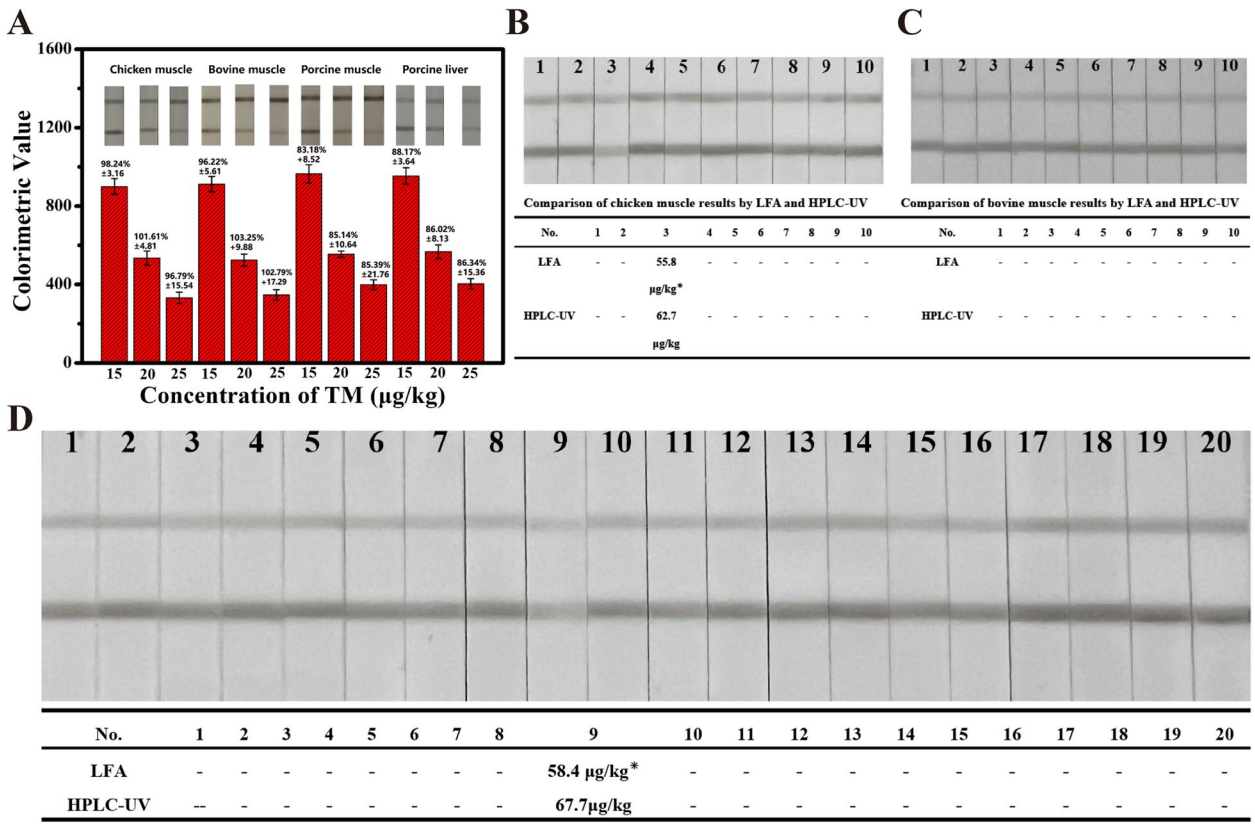

Fig. 4 **A** Sample recovery determination ($n = 3$). **B** Performance of the LFA with 10 chicken muscle samples. **C** Performance of the LFA with 10 bovine muscle samples. **D** Performance of the LFA with 20 porcine muscle samples. Note:—negative; *Further diluted 2-times for detecting by the developed LFA; The number of 1–20 indicates the sample number

atomic charge and ESP descriptors of molecules via GaussView 5.0 software (Gaussian, Wallingford, CT, USA). The molecules overlap results and 2D similarity were generated by Discovery Studio software 2019 (Dassault Systèmes, BIOVIA Corp., San Diego, CA, USA).

Synthesis of hapten and complete antigens

TM-HS was synthesized as previously described [26]. Briefly, TM (174 mg, 0.19 mmol), succinic anhydride (20 mg, 0.2 mmol), and DMAP (2.4 mg, 0.2 mmol) were dissolved in 5 mL of CH_2Cl_2, and stirred for 6 h at 25 ℃. The reaction mixture was dried by rotary evaporation and the residue obtained was identified as the hapten TM-HS.

For coupling TM-HS with the carrier protein using the active ester method [27], TM-HS (49.8 mg, 0.05 mmol), DCC (12.4 mg, 0.06 mmol), NHS (6.9 mg, 0.06 mmol) were dissolved in 3 mL DMF, and stirred at 25 ℃ for 12 h. Subsequently, KLH (40 mg) or BSA (110 mg) was dissolved in 10 mL of PBS (0.01 mol/L, pH 7.4), and the activated hapten was dropwised into the KLH or BSA solution. The mixture was stirring at 4 ℃ for 12 h, followed by dialysis in 2 L of PBS buffer (0.01 mol/L, pH 7.4) for 3 days, resulting in TM-HS-KLH/BSA.

Preparation of mAbs

Twelve female Balb/c mice (20 g, 6 ~ 8 weeks old) were immunized with TM-HS-KLH as described previously [28]. After the third immunisation, antiserum was collected and characterised by icELISA. The mice with the highest antibody titres and IR were scarified for cell fusion. The IR was calculated according to the following formula:

$$\text{Inhibition ratio } (\%) = (1 - B/B_0) \times 100\% \quad (1)$$

where B is the absorbance value of TM standard and B_0 is the absorbance value of blank.

Cell fusion, mAbs acquisition and purification were conducted as previously described [20]. The performance of mAb was evaluated based on IC_{50} using icELISA.

LFA development

Preparation of labelled-mAb

ACNPs were conjugated to mAb (ACNPs-mAb) through electrostatic adsorption as previously described with some modifications [29]. Briefly, 10 μg of mAb 12C8 was added to 1 mL of ACNPs solution and incubated for 25 min at 25 ℃. Thereafter, the obtained ACNPs-mAb was blocked with 20 μL of 10% BSA. After centrifuging at 8500 g for 10 min at 4 ℃, the pellet was ACNPs-mAb and resuspended in 10 mM PBS containing 1% glucose, 0.05% polyethylene glycol 20,000, 1% BSA, and 0.02% procline 300. As a control, CGNPs were coupled to the antibody (CGNPs-mAb) through electrostatic adsorption, consistent with previous reports [20].

Assembly of LFA

The composition of LFA strips included a sample pad, NC membrane, and an absorption pad. NC membrane was coated with 0.3 mg/mL TM-HS-BSA for the Test-line (T-line) and 0.6 mg/mL goat anti-mouse antibodies for the Control-line (C-line). Subsequently, the sample pad, NC membrane and absorbent pad were attached to the PVC backing plate in turn, cut into 3.2 mm wide strips and stored dry at 25 ℃.

Analysis of LFA performance

A 3 μL of ACNPs-mAb 12C8 was combined with sample extract of 200 μL in a 96-well plate. After 3 min incubation, strips were inserted into wells for 8 min. Qualitative analysis was performed instantly through visual inspection by naked eye, while quantitative analysis was conducted using an immunochromatographic reader. The performance of CGNPs-LFA was using the same procedure as ACNPs-LFA.

Sensitivity and specificity analysis of LFA

The sensitivity of LFA was evaluated using standard curves of TM. For qualitative analysis, the cut-off value was defined as the minimum TM concentration that caused the colour to disappear from the T-line. For semi-quantitative analysis, the intensity of T-lines was measured. TM was quantified using calibration curves (colorimetric value versus TM concentration). The IC_{50} value was defined as the concentration of TM leading to 50% inhibition of the maximum colorimetric value.

In order to evaluate the specificity of LFA, other analogues, such as 100 ng/mL of tylosin, tiamulin, avermectin, streptomycin, erythromycin and spiramycin, were detected and CR was calculated.

Sample preparation for LFA detection

Milk samples were detected directly without treatment. For chicken muscle, bovine muscle, porcine muscle and porcine liver, 1 g of each homogenised sample was added to a 10 mL polypropylene centrifuge tube. For the extraction of chicken muscle, bovine muscle and porcine liver samples, 5 mL of 80 mM citrate buffer containing 10% acetonitrile was employed, and porcine muscle was extracted using 5 mL of 80 mM citrate buffer containing 12% acetonitrile. The mixtures were vortexed for 3 min and centrifuged at 5000 g for 10 min. Finally, 0.1 mL of supernatant was diluted eightfold by PBST, and analysis by the developed LFA.

Accuracy and precision of LFA for different samples

Accuracy [expressed as recovery] and precision [expressed as coefficient of variation (CV)] of the developed LFA were determined by spiking TM at concentrations of 15, 20 and 25 μg/kg in samples. LOD was calculated according to the following equation:

$$\mathrm{LOD} = (\overline{X} + 3\ \mathrm{SD}) \times \text{dilution factor} \quad (2)$$

where $\overline{X}$ is the mean value of the measured concentrations of the 20 blanks, and SD is the standard deviation. The dilution factor was 40-fold.

Analysis of the practicality of LFA

The samples were prepared by the national standards of the People's Republic of China (Announcement No. 1163 of the Ministry of Agriculture- 6- 2009) [30]. HPLC-UV screening of TM was performed using a UltiMate 3000 HPLC system equipped with a triple-quadrupole variable wavelength detector. A C18 column (250 mm×2.1 mm, 3.5 μm) was applied for the chromatography step. The mobile phases consisted of 0.1% formic acid/water (A) and acetonitrile (B). An isocratic elution program was performed with a flow rate of 1 mL/min as follows: 70% A and 30% B. The sample injection volume was 20 μL. The UV wavelength was 286 nm.

Ten chicken muscle, 10 bovine muscle and 20 porcine muscle samples were purchased from local cattle farms and subjected to detection by HPLC-UV. In addition, those muscle samples were also tested using the developed LFA. The practicability of LFA was verified by the correlation between the HPLC-UV and LFA.

Abbreviations

TM	Tilmicosin
TYL	Tylosin
MRLs	Maximum residue limits
ELISA	Enzyme-linked immunosorbent assay
LFA	Lateral flow immunoassay
mAb	Monoclonal antibody
IC_{50}	Half maximal inhibitory
CR	Cross-reactivity
ACNPs	Amorphous carbon nanoparticles
CGNPs	Colloidal gold nanoparticles
LODs	Limits of detection
HPLC-UV	High-performance liquid chromatography-UV detector
ESP	Electrostatic potential energy
IR	Inhibition rates
CV	Coefficient of variation
DMF	N, N-dimethylformamide
DCC	N, N-dicyclohexylcarbodiimide
DMAP	4-Dimethylaminopyridine
TMB	Tetramethylbenzidine
KLH	Keyhole limpet hemocyanin
BSA	Bovine serum albumin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44280-023-00032-w.

Additional file 1: Figure S1. The UV-visible absorption spectra of TM-HS, KLH, BSA. **Figure S2.** (A) The HPLC chromatogram of standard solution of TM (concentration with 125, 250, 500, 1000, 2000 and 4000 ng/mL, respectively). (B) The HPLC analysis results for blank chicken muscle. (C) NO.3 of chicken muscle. (D) Blank bovine muscle. (E) Blank porcine muscle. (F) NO.9 of porcine muscle, respectively. **Table S1.** Immunization results. **Table S2.** Recovery and coefficient of variance for TM in spiked samples by the developed LFA ($n = 3$).

Authors' contributions

Y.W. and Q.L. contributed to this work equally and should be regarded as co-first authors. Y.W. contributed to the investigation, formal analysis and writing-original draft. Q.L. contributed to the validation and formal analysis. Z.L. contributed to the formal analysis. L.S., X.H., Y.M. and T.B. were in charge of project administration. D.P. provided the software. X.Z. contributed to the conceptualization, project administration, investigation, supervision, funding acquisition, writing-review & editing. All authors read and approved the final manuscript.

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Availability of data and materials

Data will be shared upon request by the readers.

Declarations

Ethics approval and consent to participate

The animals were acquired from the Zhengzhou Huaxing Laboratory Animal Farm and provided by the Henan Provincial Department of Science and Technology (SCXK 2019–0002). The animal experimental protocol received approval from the Scientific Ethics Committee of Henan Agricultural University (No. HNND2023031028).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. Author Dapeng Peng is a member of the Editorial Board for One Health Advances and he was not involved in the journal's review or decisions related to this manuscript.

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