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Impact of Immunogen Structure to Specificity of Fluoroquinolones Detection by Microplate and Lateral Flow Immunoassay Techniques

NADEZHDA A. TARANOVA[1], ELENA A. ZVEREVA [1,], ILIYA A. SHANIN [2] , SERGEY A. EREMIN [2] , ANATOLY V. ZHERDEV [1] AND BORIS B. DZANTIEV [1]*

[1]A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, 33 Leninsky prospect, Moscow 119071, Russia.
[2]M.V. Lomonosov Moscow State University, Chemical Department, Leninskie Gory, 119991 Moscow, Russia.
*Corresponding author E-mail: dzantiev@inbi.ras.ru

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ABSTRACT

When immunodetection of structurally related compounds important to be able to vary the selectivity of their immune recognition that, depending on the task, to identify well-defined or the total content of the compounds. The selection of immunogenic fluoroquinolone derivatives and a method for fluoroquinolone conjugation with a carrier protein allowed for modulating the specificity of the produced antibodies. We used different derivatives to produce polyclonal antibodies and studied their specificity in microplate immunoenzyme and lateral flow immunochromatographic assays. The use of hapten with free variable part for the production of antibodies allowed the detection of levofloxacin, its chiral isomer ofloxacin, and structurally related marbofloxacin. The modified hapten with free constant part allows us to develop analytical systems specific to ciprofloxacin that also have broad specificity for eight other fluoroquinolones. Comparison of the data of ELISA and LFTS showed a fundamental similarity in recognizable derivatives. However, a measure of cross-reactivity was matched.

Key words: Fluoroquinolones; ELISA; Immunochromatographic assays; Colloidal gold; immunogen structure.

INTRODUCTION

Fluoroquinolones (FQs; Fig. 1) are widely used in veterinary medicine as active antibiotic agents against gram-negative pathogens for the treatment of diseases as well as preventive measures[1, 2]. In addition, FQs are used as growth promoters of animal body weight, pathogen destroyers of drinking water sources, protective phytopathogenic agents in crop production, and in fodder and food[3]. With such wide use, FQs may enter the human body not only in the treatment of diseases, but also with foodstuffs, which gives rise to antibiotic-resistant strains of bacteria that cause toxic and allergic effects, dysfunction of the gastrointestinal tract, renal insufficiency, and deformation of mucous tissues[4]. Studies show that the emergence of fluoroquinolone antibiotic resistance is due to the use of these antibiotics for clinical, veterinary, and agricultural purposes[5, 6]. It is therefore important to determine whether FQs and other veterinary antibiotics are present in foodstuffs

Despite having a wide variety, FQs combine a number of actively used compounds.

Veterinary science uses medicines based on enrofloxacin, flumekvin, lomefloxacin, ofloxacin, ciprofloxacin, orbifloxacin, difloxacin and marbofloxacin. Depending their relative toxicity and intensity of applications, different regulastions sate necessity to control different lists of the most dangerous compounds. Thus, many countries have introduced fluoroquinolone maximum residue levels (MRL) for various foods[2]. Besides, detailed analysis of contamination should be based on much more specific detection with the recognition of individual compounds instead of total sum of different FQs.

In accordance with the regulations of the Russian Federation, MRLs are established for flumequine, danofloxacin, difloxacin, marbofloxacin, and the total content of antibiotics ciprofloxacin, enrofloxacin, ofloxacin, and norfloxacin (Table 1). Others FQ derivatives are used much less frequently but are also not safe. In accordance with the Russian legislation and the regulations of the European Union and China, MRLs for FQs are the same for all listed antibiotics. Instead of using the total content of the four abovementioned FQ antibiotics, enrofloxacin is controlled in China and the EU countries (MRL of 100 ng/mL).

Thus, two tasks are actually needed for food safety control — determination of (1) individual representatives of the FQs and (2) the total content of FQs. Immunochemical detection methods are considered promising due to their high specificity and sensitivity.

The authors primarily seek broad specificity, but also that it does not reach. It is interesting also to be able to detect a limited number of compounds, for it is a narrow selective analysis or as an addition to the under-selective. In this respect of fundamental importance to point selection and modification of the modifiable FQ[7]. There are several known methods for producing immunogens varying in haptene modifications[8, 9]. In ref. Suryoprabowo, S., et. Al[10], monoclonal antibodies were produced based on a protein conjugate with modified ciprofloxacin. The developed ELISA method allowed determination of nine FQs antibiotics (100% to 3.5%). In another study by Wang Z.H., et. al., ELISA was utilized to detect four FQs antibiotics (100% to 7%)[11] based on the antibodies produced using the derivative enrofloxacin. Researchers are seeking to implement test systems with wide specificity, but realize a test systems for determining sum of not more than 10 compounds.

The purpose of this study was to develop immunoassays differing in specificity to FQs due to using differing immunogens. We also compared the enzyme-linked immunosorbent assay (ELISA) and lateral flow test systems (LFTS) in terms of their quantitative parameters of speficity. These two approaches are highly actual for different analytical purposes. The merits of ELISA include high sensitivity, possibility of simultaneous characterization of up to 40 samples (in two repetitions). Unlike ELISA, the LFTS method allows rapid identification of compounds in field conditions. The both of the systems are based on competitive binding of antibodies with free and immobilized haptens. The question of the influence of the different concentrations of the immunoreactants in ELISA and LFTS on the quantitative parameters of the cross-reactivity immunoassay systems very poorly covered in the scientific literature.

MATERIALS AND METHODS

Chemicals

Goat anti-rabbit immunoglobulin antibodies were purchased from Arista Biologicals (Allentown, PA, USA). Polyclonal rabbit anti-FQ antisera and their IgG-fraction were obtained as described in[12, 13]. Peroxidase-labeled anti-rabbit immunoglobulins were from the N.F. Gamaleya Institute of Microbiology and Epidemiology (Moscow, Russia).

Sodium azide, bovine serum albumin (BSA), ovalbumin (OVA), 3,3´,5,5´-tetramethylbenzidine (TMB), dimethyl formamide (DMF), formaldehyde, and Tween-20 were from Sigma (St. Louis, MO, USA). Gold chloride, levofloxacin (Lev), ciprofloxacin (Cip), marbofloxacin (Mar), flumekvin (Flu), danofloxacin (Dan), enrofloxacin (Enro), ofloxacin (Ofl), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide

(NHS), triethylamine were from Fluka (Buchs, Switzerland). All other chemicals (salts and solvents of analytical grade) were from Chimmed (Moscow, Russia).

All solutions for syntheses were prepared using purified water obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Stock solutions of FQs (1 µg/mL) in water were stored at -20 °C.

Mdi Easypack (Advanced Microdevices, Ambala Cantt, India) membrane kits were used to manufacture immunochromatographic tests. They included a plastic support, working nitrocellulose membrane CNPC with a 12 µm pore size, GFB-R4 separation membrane, "-R7 glass fiber membrane, and an 045 adsorption membrane. Costar microplates 9018 (Corning, NY, USA) were used for ELISA.

Milk samples (3.2% fat) were purchased from the local market. Spiked milk samples were prepared by mixing the analyte stock solutions and pre-tested antibiotic-free milk samples.

Synthesis of fluoroquinolone-protein conjugates

Derivate of ciprofloxacine (derCip; see Fig. 2) was synthesized by V.A. Popova (M.V. Lomonosov Moscow State University, Russia) as described by Huet et al[14]. Carboxyl groups of Lev or derCip were activated via carbodiimide chemistry. FQ (14.7 µmol), EDC (5.7 mg; 30 mmol), and NHS (3.5 mg; 30 µmol) were dissolved in 1.0 mL of DMF and incubated for 2 h at room temperature under stirring. 10 mg of OVA were dissolved in 8 mL of 50 mM carbonate buffer, pH 9.5, with added 50 µL of triethylamine and incubated for 1 h at 4 °C under stirring. A FQs solution with activated carboxyl groups was slowly added dropwise to the protein solution while stirring. The mixture was incubated for 5 h with RT in darkness. The synthesized conjugate was separated from the low molecular substances by dialysis against distilled water for 5 d with a daily change. The last two dialysis processes were conducted against 0.01 M phosphate saline buffer solution, pH 7.4 (PBS). The solution was divided into 1 mL aliquots and stored at -20 °C until use

ELISA of fluoroquinolones

The Lev-OVA or derCip-OVA conjugates were added in microplate wells (1 µg/mL in PBS, 100 µl per well) and incubated overnight at 4 °C. After 4 times washing with PBST, the stock antibiotic solutions were diluted in PBS containing 0.05% Triton X-100 (in PBST) to obtain a series of solutions in the range of 50 to 0.001 ng/mL for Levofloxacin and 1000 to 0.14 ng/mL for Ciprofloxacin and added to the microplate wells (50 µL per well). The antibodies were diluted in PBST to obtain a concentration of 6 ng/mL for Abs/Levofloxacin and 9 ng/mL for Abs/Ciprofloxacin (50 µL per well), and the microplate was incubated for 1 h at 37 °C. After 4 times washing with PBST, peroxidase-labeled anti-rabbit antibodies were added (dilution of the commercial product in PBST was 1:6,000, 100 µL per well) and incubated for 1 h at 37 ºC. Finally, the microplate was washed three times with PBST and once with distilled water. To detect activity of the bound peroxidase label, a substrate solution containing 0.42 mM TMB and 1.8 mM H_2O_2 in 0.1 M citrate buffer, pH 4.0, was added (100 µL per well).

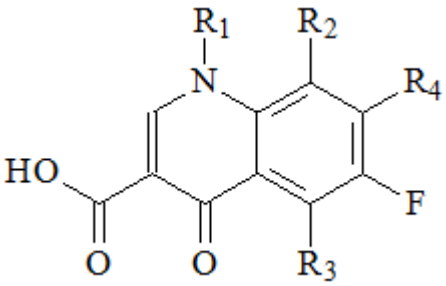

Fig. 1: General structural pattern of fluoroquinolones (R_4 – variable region)

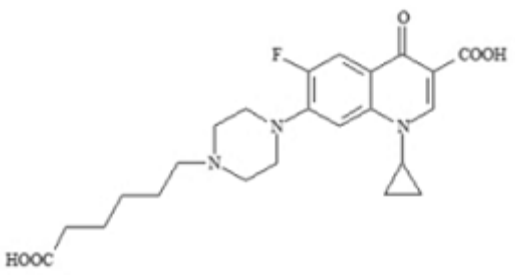

Fig. 2: Structure of the derivate of ciprofloxacine $C_{22}H_{30}O_4N_3F$

After 15 min incubation at room temperature, the reaction was stopped with 1 M H_2SO_4 (50 µL per well). The optical density of the reaction product at 450 nm was measured using a microplate reader ZENYTH 3100 (Anthos Labtec Instruments, Salzburg, Austria).

Preparation of gold nanoparticles

Gold nanoparticles (GNPs) were prepared according to Frens[15]. Briefly, 1.0 mL of a 1% water solution of $HAuCl_4$ was added to 97.5 mL of water. The mixture was heated to reflux, and 1.5 mL of 1% sodium citrate solution was added. After refluxing for 30 min, the preparation was cooled and then stored at 4 °C.

Conjugation of antibodies with gold nanoparticles

The protocol proposed by Byzova et al[16] was used to choice antibody concentration for the conjugation. A series of aqueous solutions of antibodies in concentrations ranging from 0.5 to 200 µg/mL was prepared. One hundred microliters of each solution was mixed with 1.0 mL of the GNP preparation (D_{520} = 1.0). The mixture was incubated and stirred for 10 min at room temperature. Then, 0.1 mL of a 10% NaCl solution was added to each preparation. D_{520} was measured after 10 min of stirring at room temperature. Basing on the selected antibodies concentration (see Results and Discussion), the GNPs-antibodies conjugate was prepared as described below.

The antibodies were dialyzed against 10 mM Tris-buffer, pH 8.5. 0.1 M potassium carbonate was added dropwise to the GNP solution (D_{520} = 1.0) until it reached pH 8.5, and then the antibodies were added. The mixture was incubated for 30 min with stirring at room temperature. After this, BSA solution (10%) was added to a final concentration of 0.25%. The GNPs were separated from unbound molecules by centrifugation at 13,000 g for 15 min at 4 °C. The pellets were resuspended in 50 mM Tris buffer, pH 7.4, containing 0.25% BSA. For long-term storage at 4 °C, 0.05% sodium azide was added to the conjugates.

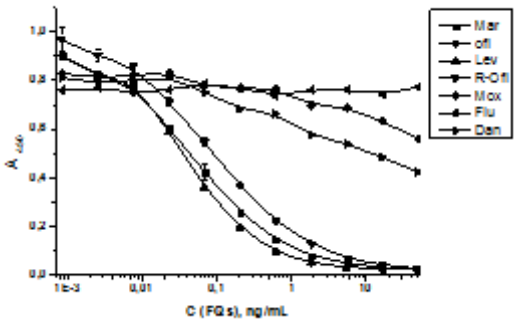

Fig. 3: Competitive curves for levofloxacin and other FQs detection by ELISA

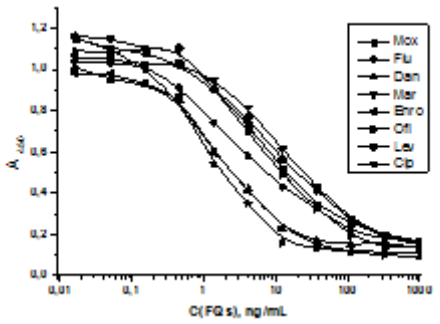

Fig. 4: Competitive curves for ciprofloxacin and other FQs detection by ELISA

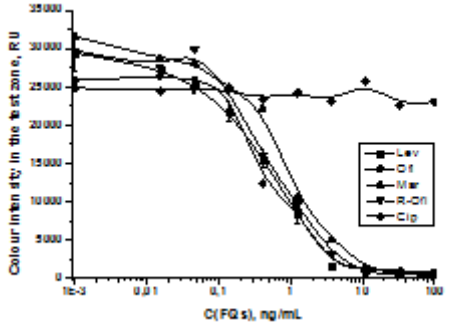

Fig. 5: Competitive curves for levofloxacin and other FQs detection

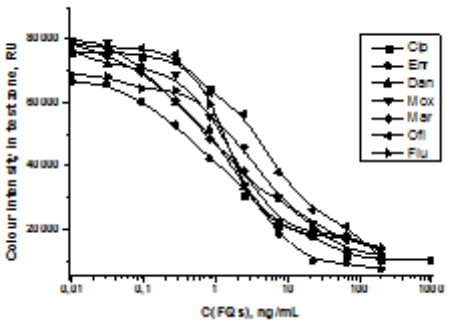

Fig. 6: Competitive curves for ciprofloxacin and other FQs detection

Size characterization of the gold nanoparticles and their conjugates with antibodies

To characterize the particle size, images of the GNPs and their conjugates were obtained with a transmission electron microscope (CX-100, Jeol, Tokyo, Japan) at an accelerating voltage of 80,000 V and a magnification of 3,300,000[16].

A Zetasizer Nano ZSP (Malvern, UK) particle-size analyzer was used to estimate the hydrodynamic diameter of free nanoparticles and their conjugates. Measurements were carried out at a 103° angle of light dispersion with preliminary temperature control of the sample up to 20 °C. The sample volume was 70 µL. Each sample was subjected to 10 measurements.

Determination of the amount of antibodies adsorbed on the surface of GNP

To quantify the antibodies bound, the following ELISA protocol was used[17]. The of Lev-BSA and derCip-OVA conjugates were immobilized in microplate wells (5 µg/mL in PBS, 100 µl per well) and incubated overnight at 4 °C. After washing with PBST, supernatants of the CNP-Antibodies conjugate (*see section 2.5*) were titrated with period 3. Also the antibodies against antibiotics were diluted in PBST to obtain a series of solutions in the concentration range of 10 µg/mL to 0.1 ng/mL. Antibody solutions and supernatants from centrifugation were added to the microplate wells (50 µL per well), and the microplate was incubated for 1 h at 37 °C. Further analyses were conducted using the method described below (see section 2.3). The color intensity dependence of the substrate reaction product with HRP after concentrating the antibodies that interact with the immobilized conjugates of Lev-BSA and derCip-OVA was obtained.

The amount of antibodies in the supernatant was determined by comparing the calibration curve and the curve of untitrated supernatant. The amount of antibodies adsorbed

Table 1: Analytical characteristics of the developed test systems

Test system	Detection limit, ng/mL	Operating range, ng/mL	IC_{50}, ng/mL
ELISA Lev	0.004	0.01–0.2	0.04
ELISA Cip	0.14	0.3–3.6	1.03
LFTS Lev	0.1	0.2–1.6	0.44
LFTS Cip	0.05	0.1–3.8	0.70

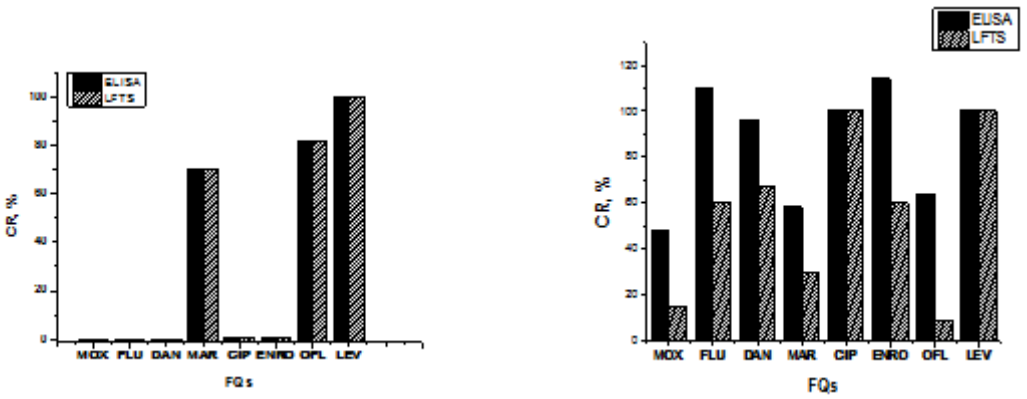

Fig. 7: ELISA and LFTS specificity using the antibodies against levofloxacin (a) and the antibodies against ciprofloxacin (b).

on the surface of GNPs was determined by the difference of antibodies added during conjugation and those found in the supernatant.

Fabrication of lateral flow tests

Immunoreagents were immobilized on membranes using an IsoFlow automated dispenser (Imagene Technology, Hanover, NH, USA). The test zone was formed by Lev-OVA or derCip-OVA conjugates, and the control zone was formed by the goat anti-mouse immunoglobulin. The following concentrations and immobilization conditions were used: Lev-OVA and derCip-OVA conjugates, 1.0–2.0 mg/mL in PBS; goat anti-rabbit immunoglobulins, 1 mg/mL in PBS. One microliter of all loading solutions was applied per cm of strip width. The GNPs-antibodies conjugate (at a dilution corresponding to D_{520} = 4.0) was applied onto a glass fiber membrane, which was then dried at room temperature for at least 20 h. The conjugate load was 32 µL per 1 cm of strip width. After the dispensing process, the membranes were dried at room temperature for at least 20 h.

After the membrane components were assembled, they were cut with an Index Cutter-1 (A-Point Technologies, Allentown, PA, USA) into 3.5 mm width strips. The test strips were hermetically packed in laminated aluminum foil bags containing silica gel as the desiccant with the use of a FR-900 mini-conveyor. The cutting and packing were carried out at 20–22 °C in a special room with a relative humidity less than 30%. The packed test strips were stored at room temperature.

Immunoassay procedure

The immunochromatographic assay was performed at room temperature. Pure and spiked milk samples were diluted by 20% with PBS (milk:PBS ratio is 80:20). A lateral flow test strip was vertically submerged into an analyte solution or a milk sample for 10 min, which corresponded to the time required for the fluid front to migrate through the entire length of the working membrane.

The binding of the label in the test and control zones was recorded with the use of a CanoScan LiDE 90 scanner (Canon, Tokyo, Japan) followed by digital processing of the images by TotalLAB v2009 (Nonlinear Dynamics, Newcastle, UK). This program was used to determine the line boundaries, total the intensities of all pixels belonging to a particular unit, and normalize the sums to the line surface area, thus representing the color intensity in relative units (RU).

Based on the color intensities (Y) for different concentrations of the analytes (x), calibration curves were constructed using the four-parameter sigmoid function[18]:

$$Y = ((A - D)/(1 + (x/C)^B)) + D \quad ...(1)$$

where is the asymptotic maximum (the color intensity in the absence of the analyte), is the slope of the curve in semilogarithmic coordinates at the inflection point, ! is the concentration of the analyte at the inflection point, and D is the asymptotic minimum (the intensity of the background coloration).

As recommended by Sittampalam et al[19], the quantitative limit of detection was calculated as the amount of antibiotic that corresponded to 10% binding inhibition. Lower and upper limits of the working range were calculated as amounts of hapten that caused 20% and 80% binding inhibition, respectively.

Cross reactivity (CR) was determined using the next equitation:

$$CR = \frac{IC_{50\text{detected FQ}}}{IC_{50\text{ cross reacted FQ}}} \cdot 100\% \quad ...(2)$$

where IC_{50} is analyte concentration leading to 50% inhibition of antibody binding.

RESULTS AND DISCUSSION

Development of the presented analytical methods involved the following steps: preparation and characterization of immunoreagents (FQs-OVA conjugates, GNP, and GNP conjugates with the antibodies against levofloxacin and ciprofloxacin); optimization of the assay conditions, and characterization of the development analytical systems. Haptene conjugates with ovalbumin were synthesized via carbodiimide chemistry. The molar antibiotics:protein ratio in the synthesis was 67:1.

Comparative analysis of the absorption spectra of free antibiotics and proteins with spectral data of the conjugates showed that immunoreagents obtained by this method contained both the carrier protein and antigen.

ELISA development

All FQ molecules have constant and variable regions. Thus, to generate antibodies with group specificity, animals have to be immunized with conjugates of FQ, which variable region groups are bound with protein-carrier. There are several ways to implement this approach, including the use of available active groups of certain FQs (primary and secondary amino groups) and the modification of variable region structures (introduction of active groups, usually carboxyl or amino groups, without stem or through the stem, which is commonly several methylene groups)[7, 8].

To implement the analysis with narrow specificity was requested to use as an immunogen the conjugate of Levofloxacin with BSA, where FQ was modified by the invariable part. To implement the analysis with wide specificity was requested to use as an immunogen the conjugate of Ciprofloxacin with BSA, where FQ was modified by the variable part using a ciprofloxacin derivative containing 5-carboxypentyl.

Chosen optimal conditions of the ELISA assay procedure provide maximum sensitivity and accuracy. The ELISA based on the antibodies against levofloxacin is characterized by a working range of 0.01–0.2 ¼g/mL with a detection limit of 0.004 ng/mL and IC_{50} (concentration causing 50% inhibition of binding) of 0.04 ng/mL (Fig. 3).

The development of the ELISA system based on the antibodies against ciprofloxacin showed that the introduction of the bridge between hapten and the carrier molecule caused reduction of non-specific binding with ovalbumin. Conjugates were used in the developed systems comprising ciprofloxacin, a modified 6-bromohexane acid.

The ELISA based on the antibodies against ciprofloxacin is characterized by a working range of 0.30–3.58 ng/mL with a detection limit of 0.14 ng/mL and IC_{50} of 1.03 ng/mL (Fig. 4).

LFTS development

GNPs were synthesized by reduction of $HAuCl_4$ to Au^{014}. The selection of the optimum antibody concentration for GNPs conjugates synthesis was based on the method described in ref[20]. Based on the concentration dependencies (Fig. 3), the FQ antibodies were taken for conjugation in amounts two to four times higher than the point at which OD_{580} plateaus (5 ìg/mL). the antibodies against levofloxacin are in concentration of 10 ìg per milliliter of the colloidal solution and the antibodies against ciprofloxacin are in concentration of 20 ìg per milliliter of the colloidal solution. Concentrations of immunoglobulins, at which OD_{580} reaches the plateau, provided sufficient surface coverage to stabilize the particle and prevented nanoparticle aggregation caused by free surface area.

In developing the LFTS optimum concentrations of immobilized Lev-OVA and derCip-OVA conjugates, the detecting GNP conjugates with the antibodies against levofloxacin and ciprofloxacin were selected, including components of the test systems. Optimization of the test systems for detection of antibiotics in milk was based on previous experiments[16, 21, 22]. Pure and spiked milk samples were diluted by 20% with PBS (milk:PBS ratio is 80:20). Concentrations of the immobilized protein-hapten conjugates varied in the range of 0.5–2 mg/mL to provide the maximum sensitivity of the system. Optimal concentration was 1.0 mg/mL.

The optimal optical density of GNPs-antibodies conjugates was 4.0; it allowed us to obtain signals 10 times higher as compared with the visual detection threshold (1000 RU). The optimal sample preparation of milk samples consisted of sample dilution with 20% PBS to provide the maximum signal/noise ratio.

At optimal conditions the LFTS for levofloxacin was characterized by a range of detectable concentrations of 0.2–1.60 ng/mL with a detection limit of 0.03 ng/mL and IC_{50} of 0.15 ng/mL (see Fig. 5).

LFTS for ciprofloxacin using the antibodies against ciprofloxacin was characterized by a range of detectable concentrations of 0.12–3.8 ng/mL with

a detection limit of 0.05 ng/mL and IC_{50} of 0.7 ng/mL (see Fig. 6).

Analytical parameters of the development test systems are summarized in the Table 1. ELISA and LFTS systems based on the antibodies against levofloxacin are characterized by different detection limits and working ranges. Detection limit of ELISA is 25 times lower than the LFTS. ELISA and LFTS systems based on the antibodies against ciprofloxacin are characterized by the similar parameters.

The developed immunoassay test systems were used for processing spiked milk samples in "added – revealed" experiments. Sample preparation of milk samples consisted of a 20% PBS addition. The degree of levofloxacin determined using LFTS and ELISA was 98–112% and 97–107%, respectively. The degree of ciprofloxacin detected using LFTS and ELISA was 95–114% and 98–109%, respectively. Data were obtained for 5 replicates.

Comparison of ELISA and LFTS specificity

This study allowed us obtaining two FQ conjugates with BSA that were used to produce specific polyclonal antibodies. An unmodified levofloxacin derivative was used for levofloxacin-BSA synthesis by which antibodies with a narrow specificity were produced. Antibodies with broad specificity were obtained through the use of ciprofloxacin-BSA conjugates synthesized using a ciprofloxacin derivative containing 5-carboxypentyl, i.e, radical in a nitrogenous molecule base[23].

The immunoassay test systems developed have been characterized for specificity to eight FQs: levofloxacin (Lev), ciprofloxacin (Cip), marbofloxacin (Mar), flumekvin (Flu), danofloxacin (Dan), enrofloxacin (Enro), ofloxacin (Ofl), moxifloxacin (Mox). Data on specificity of the test systems based on the antibodies against levofloxacin are shown in Fig. 7a. Data on the specificity of test systems with the use of the antibodies against ciprofloxacin antibodies are shown in Fig. 7b.

As can be seen, antibodies obtained using different immunogens differed largely in specificity. Immunochemical systems based on the antibodies against levofloxacin (Fig. 8a) were characterized by a narrow specificity and were able to detect levofloxacin (100%), ofloxacin (chiral isomer) (80%), and marbofloxacin (70%). Cross-reaction of these antibodies with respect to levofloxacin and marbofloxacin was due to their similar structures, which differ in the conformation of the radical in the 7th position of the quinolone core and the use of a nitrogen atom in the marbofloxacin oxazine ring instead of a carbon atom. This aforementioned caused the cross-reaction percentage with marbofloxacin to be below 100%.

The immunochemical system based on the antibodies against ciprofloxacin (Fig. 8b) had broad specificity (>50%) to eight FQs: moxifloxacin, flumekvin, danofloxacin, marbofloxacin, ciprofloxacin, enrofloxacin, ofloxacin and levofloxacin. The ciprofloxacin molecule was characterized by greater steric mobility that allowed for the production of antibodies specific to the quinolone core because of the linker used for carrier protein conjugation.

CONCLUSIONS

Two types of immunoassay test systems were developed differing in specificity to FQ antibiotic classes. The difference in the specificity of the test systems was based on applicable antibodies derived using differing immunogens. The use of levofloxacin for antibody production allowed for detecting its chiral isomer ofloxacin and structurally related marbofloxacin. The use of a ciprofloxacin derivative containing 5-carboxypentyl allowed us to develop analytical systems specific to ciprofloxacin that also had broad specificity (>50%) to eight other FQs. In the case of using the antibodies against levofloxacin ELISA and LFTS are characterized by a narrow specificity with respect to the same FQs. Specificities of the systems based on the antibodies against ciprofloxacin are different. ELISA system based on the antibodies against ciprofloxacin allows determining the sum of 5 FQs. While LFTS based on the antibodies against ciprofloxacin allows determining all 8 FQs.

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Author Contributions

Nadezhda Taranova and Elena Zvereva contributed to the production, analysis and interpretation of the experimental results. Iliya Shanin contributed to the design of the experiments, the analysis and interpretation of the results. Sergey Eremin, Anatoly Zherdev and Boris Dzantiev had the initial idea for the study, contributed to the design of the experiments, analysis and interpretation of the results. All authors participated in the preparation of the manuscript.

REFERENCES

1. Kuhlmann, J., A. Dalhoff, and H.J. Zeiler, *Quinolone Antibacterials,* 3rd ed.;. Springer: Berlin Heidelberg, Germany, 2012; pp. 10-202.
2. Acton, Q.A., *Quinolones—Advances in Research and Application*, 1st ed.; ScholarlyPaper, UK, 2013; 48 p.
3. Seyfried, E.E., *Tetracycline Resistance Genes in Aquaculture Environments: Genotypic Diversity and Potential Resistance Reservoirs*, 1st ed.; University of Wisconsin—Madison, USA, 2007; pp. 106-185.
4. Riesbeck, K., *Effects of fluoroquinolones on the immune system,* 1st ed.; Lund University, Malmö, Sweden, 1994; 130 p.
5. Bansal, D. and V.K. Purohit, Accessibility and use of essential medicines in health care: Current progress and challenges in India. *Journal of Pharmacology Pharmacotherapeutics*, **4**(1): p. 13-18 (2013).
6. Ronald, A.R. and D. Low, Fluoroquinolone Antibiotics, Birkhäuser, Basel, Germany, 2012; 261 p.
7. Burkin, M. A., Galvidis, I. A. Improved group determination of tetracycline antibiotics in competitive enzyme-linked immunosorbent assay. *Food and Agricultural Immunology*, **20**(3),: p. 245-252 (2009).
8. Staimer, N., S.J. Gee, and B.D. Hammock, Development of a class-selective enzyme immunoassay for urinary phenolic glucuronides. *Analytica Chimica Acta*, **444**(1): p. 27-36 (2001).
9. Pastor-Navarro, N., et al., Development of a group-specific immunoassay for sulfonamides: Application to bee honey analysis. *Talanta*, **71**(2): p. 923-933 (2007).
10. Suryoprabowo, S., et al., Development of a broad specific monoclonal antibody for fluoroquinolone analysis. *Food Analytical Methods*, **7**(10): p. 2163-2168 (2014).
11. Wang, Z.H., et al., Development of a highly sensitive and specific immunoassay for enrofloxacin based on heterologous coating haptens. *Analytica Chimica Acta*, **820**: p. 152-158 (2014).
12. Shanin I.A., et. al., Determination of levofloxacin (the Levorotatory Stereoisomer of Ofloxacin) in milk by an indirect enzyme-linked immunosorbent assay. *Moscow University Chemistry Bulletin*, **69**(3): pp. 136-141 (2014).
13. Shanin I.A., et. al., Determination of the antibiotic fluoroquinolone levofloxacin in urine by fluorescence polarization immunoassay. *Journal of Analytical Chemistry*, **70**(6): p. 712-717 (2015).
14. Huet A.-C., et. al., Simultaneous determination of (fluoro)quinolone antibiotics in kidney, marine products, eggs, and muscle by enzyme-linked immunosorbent assay (ELISA). *Journal of Agricultural and Food Chemistry*, **54**: pp. 2822-2827 (2006).
15. Frens, G., Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature Physical Sciences*, **241**: 20–22 (1973).
16. Byzova, N.A., et al., Rapid pretreatment-free

immunochromatographic assay of chloramphenicol in milk. *Talanta*, **81**(3): p. 843-848 (2010).
17. Sotnikov D.V., A.V. Zherdev, and B.B. Dzantiev, Development and application of a label-free fluorescence method for determining the composition of gold nanoparticle–protein conjugates. *International Journal of Molecular Sciences*, **16**(1): p. 907-923 (2015)
18. Kurganov, B.I., et al., Criterion for Hill equation validity for description of biosensor calibration curves. *Analytica Chimica Acta*, **427**(1): p. 11-19 (2001).
19. Sittampalam, G.S., et al., Application of experimental design techniques to optimize a competitive ELISA. *Journal of Immunological Methods*, **160**(2): p. 151-161 (1996).
20. Byzova, N.A., et al., Immuno chromatographic technique for express determination of ampicillin in milk and dairy products. *Applied Biochemistry and Microbiology*, **47**(6): p. 627-634 (2011).
21. Safenkova, I.V., A.V. Zherdev, and B.B. Dzantiev, Correlation between the composition of multivalent antibody conjugates with colloidal gold nanoparticles and their affinity. *Journal of Immunological Methods*, **357**(1-2): p. 17-25 (2010).
22. Byzova, N.A., et al., Rapid immunochromatographic assay for ofloxacin in animal original foodstuffs using native antisera labeled by colloidal gold. *Talanta*, **119**: p. 125-132 (2014).
23. Tittlemier, S., et al., Development of a direct competitive enzyme-linked immunosorbent assay for the detection of fluoroquinolone residues in shrimp. *Food Analytical Methods*, **1**(1): p. 28-35 (2008).