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Rapid pretreatment-free immunochromatographic assay of chloramphenicol in milk

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ABSTRACT

A pretreatment-free immunochromatographic assay for detection of chloramphenicol (CAP) in milk was developed. The assay is based on competition between CAP molecules in the sample and immobilized CAP–protein conjugate for binding to monoclonal anti-CAP antibodies conjugated with colloidal gold particles (average diameter 30 nm). The assay is carried out in the course of sample flowing along test strip with immobilized reactants, and its results can be detected by the naked eye or by a photometric device. Effect of the concentration of immunoreactants on assay characteristics was studied. The assay protocol with maximal sensitivity and reliability was optimized using measured values of brightness of lines. Detection limit for CAP is 10 ng mL^{-1}. Assay duration is 10 min, and it can be carried out at room temperature without any additional devices and reactants. The developed test strip has been applied to CAP detection in dairy products.

1. Introduction

Antibiotics are intensively used both in the medical and veterinary practice. However, their therapeutic action can be accompanied by a number of undesirable effects such as disbacteriosis, allergic reactions, suppression of certain enzymes, etc. On top of that, excessive use of antibiotics may lead to selection of antibiotic-resistant strains. Consequently, monitoring of antibiotics in food is extremely important task [1–4].

Chloramphenicol (CAP) is one of the most widely used antibiotics with comparatively slow elimination rate. Maximal CAP content varies substantially for different countries and foodstuffs. Thus, a maximum residual permisable limit (MRPL) of 0.3 ng g^{-1} in foodstuffs has been established for CAP in EU countries [5,6] and also in New Zealand in 2008 [7]. The maximum residue level (MRL) of CAP in Russia for milk, meat and eggs is 10 ng g^{-1} [8,9]. Comparison of the CAP content with its MRL is a necessary element in quality control of both row and commercial milk. Thus, efficient analytical methods need to become available to allow for extensive monitoring in the dairy industry.

A number of methods for CAP detection in foodstuffs have been developed including microbiological assays [10,11], liquid and gas chromatography [12–17]. Among different analytical techniques, immunochemical detection methods are considered promising due to their high specificity and sensitivity [10]. Known immune techniques for CAP detection include ELISA [18,19], chemiluminescent enzyme immunoassay [20,21], immunosensor [22,23] and small-molecule microarray [24]. ELISA kits for chloramphenicol manufactured by Ridascreen (R-Biopharm AG), CAP EIA (Eurodiagnostica) and others are now available on the market. However, ELISA is a comparatively time consuming technique (the assay duration is in the range of 1–2 h) and requires special registering equipment. Thus, developing rapid, out-of-laboratory techniques for monitoring CAP in milk is extremely important.

Immunochromatographic analysis is a promising solution to the problem of rapid CAP monitoring. It is based on the use of membrane carriers (test strips) with immobilized immunoreactants [25,26]. In this assay, contact of the test strip with the sample initiates movement of reactants along the membrane, followed by immunochemical reactions and formation of easily detectable colored lines.

To date, two papers that describe immunochromatographic assays of CAP have been published. The system of Jang et al. [27] is designed for analysis of plasma, tissues, and milk. The system of Li et al. [28] has been developed for analysis of fish (*Nile tilapias*) tissues. However, both systems require pretreatment of samples and therefore are not suitable for field testing.

Research described here focuses on the creation of immunochromatographic test for rapid and simple monitoring of CAP in milk that overcomes the above-stated restrictions.

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The assay is based on video-digital registration of the color change. Quantitative criteria were used to optimize the assay conditions in order to reach the necessary sensitivity. This paper presents the data on the assay development and its application for CAP content analysis in milk.

2. Materials and methods

2.1. Chemicals

Chloramphenicol, chloramphenicol succinate sodium salt (CAP-Su), gentamicin, streptomycin, amoxicillin, ampicillin, penicillin V, penicillin G, cloxacillin, bovine serum albumin (BSA), 3,3′,5,5′-tetramethylbenzidine (TMB), sodium azide, glycerol, Triton X-100 and Tween-20 were from Sigma, USA. Soybean trypsin inhibitor (STI), 1-cyclohexyl-3-(2-morpholino-ethyl)carbodiimide metho-p-toluence sulphonate (CCMS), N-hydroxysuccinimide (NHS), N,N-dimethylformamide (DMF) were from MP Biomedicals, USA. Gold chloride was from Fluka, Switzerland. Monoclonal antibody against CAP was from Meridian Life Science, USA (G35222M). Goat, rabbit and sheep anti-mouse immunoglobulins (GAMIss, RAMIss and SAMIss, correspondingly) were from Imtek, Russia. Peroxidase-labeled anti-mouse immunoglobulins were from the Gamaleya Institute of Microbiology and Epidemiology, Russia. All other chemicals (salts and solvents of analytical grade) were from Khimmed, Russia.

Solutions of colloidal gold and its conjugates were prepared using de-ionized water (MilliQ, Millipore, USA). Stock solutions of CAP (10 mg mL^{-1}) in ethanol were stored at 4 °C. Stock solutions of other antibiotics were prepared in 50 mM sodium citrate buffer, pH 6.4, directly before analysis.

2.2. Apparatus

UV spectra were recorded using a Shimadzu UV-1201 spectrophotometer (Shimadzu, Japan). The ELISA was carried out using a WellWash 4 MK 2 washer (Thermo Electron Corporation, USA), and its results were registered by a Zenyth 3100 microplate reader (Anthos Labtec Instruments, Austria).

Electron microscopy of the colloidal gold particles was carried out using a CX-100 electron microscope (Jeol, Japan). The following equipment was used for preparation of immunochromatographic test strips: IsoFlow dispenser (Imagene Technology, USA), Index Cutter-1 (A-Point Technologies, USA), and FR-900 mini-conveyor (Wenzhou Dingli Packing Machinery Co., China).

Costar microplates 9018 (Corning, USA) were used for ELISA, and mdi Easypack (Advanced Microdevices, India) kits of membranes to manufacture immunochromatographic tests. ELISA kits "RIDASCREEN® Chloramphenicol" (R-Biopharm AG, Germany) were used for reference assay of CAP.

2.3. Preparation of hapten–protein conjugates

The CAP-Su hapten was covalently attached to BSA and STI using the NHS ester method [29] with modifications. To prepare the CAP ester, 8.5 mg (75 μmol) of NHS, 32 mg (75 μmol) of CCMS and 22 mg (50 μmol) of CAP-Su were sequentially dissolved in 1 mL of DMF and kept overnight at room temperature. Next day, 10 mg of BSA or STI was dissolved in 0.6 mL (for BSA) or 1.0 mL (for STI) of 50 mM sodium carbonate buffer, pH 9.6, and 5% (v/v) of DMF was added. The protein solutions were stored at 4 °C for 1 h and then the solution of activated CAP ester was slowly added drop wise (120 μL for BSA or 200 μL for STI solution) with vigorous stirring. The suspension was shaken for 2 h at room temperature and kept at 4 °C overnight. The conjugates obtained in this manner were dialyzed against 5 changes of 50 mM potassium phosphate buffer, pH 7.4, 0.1 M NaCl (PBS) for 3 days at 4 °C. Molar incorporation of CAP was estimated based on adsorption at 260 and 280 nm.

2.4. ELISA testing of antibodies

CAP–BSA or CAP–STI was added into the microplate wells (2 μg mL^{-1} in PBS, 100 μL per well) and incubated overnight at 4 °C. The wells were washed four times with PBS containing 0.05% Triton X-100 (PBST). Antibody dilutions (from 3 μg mL^{-1} to 50 pg mL^{-1}, 100 μL per well) in PBST were added and incubated for 1 h at 37 °C. After washing, peroxidase-labeled anti-mouse antibodies were added into the wells (dilution of the commercial product in PBST was 1:6000, 100 μL per well) and incubated for 1 h at 37 °C. The microplate was washed three times with PBST and once with distilled water. To detect bound peroxidase activity, substrate solution containing 0.42 mM TMB and 1.8 mM H_2O_2 in 0.1 M sodium citrate buffer, pH 4.0, was added (100 μL per well). After 15 min incubation at room temperature the color reaction was stopped using 50 μL of 1 M H_2SO_4. The optical density of the reaction product was measured at 450 nm using a Zenyth 3100 microplate reader (Anthos Labtec Instruments, Austria).

2.5. Competitive ELISA of CAP

CAP–BSA or CAP–STI was immobilized in the microplate wells as described above. The stock CAP solution was diluted in PBST to obtain a series of solutions in the range of 1 μg mL^{-1} to 0.2 pg mL^{-1} that were added into the microplate (50 μL per well). Alternatively, other antibiotics (gentamicin, streptomycin, amoxicillin, ampicillin, penicillin V, penicillin G, cloxacillin) diluted in PBST in the range from 1 mg mL^{-1} to 1 ng mL^{-1} were added. Subsequently 50 μL of anti-CAP antibodies (40 ng mL^{-1} for CAP–BSA and 400 ng mL^{-1} for CAP–STI, in PBST) were added into the wells, and the microplate was incubated for 1 h at 37 °C. The incubation with peroxidase-labeled anti-mouse antibodies, enzymatic reaction and photometric measurements were carried out as described above.

Dependences of the optical density (y) from the antigen concentration in the sample (x) were fitted using a four-parameter logistic function $y = (A - D)/(1 + (x/C)^B) + D$ by the Origin 7.5 software (OriginLab, USA). The value of C corresponds to the antigen concentration inhibiting 50% of maximal antibody binding (IC_{50}). The antigen concentration resulting in 10% of inhibition (IC_{10}) was calculated using the same function.

2.6. Preparation of colloidal gold particles

Colloidal gold particles with 30 nm average diameter were prepared according to the protocol described in [30]. Briefly, 1.0 mL of 1% water solution of $HAuCl_4$ was added to 97.5 mL of water. The mixture was heated to boiling and 1.5 mL of 1% sodium citrate solution was added. After boiling for 30 min the preparation was cooled and then stored at 4 °C.

2.7. Electron microscopy of the colloidal gold particles

The colloidal gold preparation was applied in chloroform to nets (300 mesh, Pelco International, USA) covered by supporting polyvinyl formal film. Images were obtained using a CX-100 electron microscope (Jeol, Japan) under accelerating voltage 80,000 V and enlargement 3,300,000.

2.8. Optimization of antibody concentration for conjugation with colloidal gold

A series of aqueous solutions of anti-CAP antibodies in concentrations ranging from 5 to 200 μg mL^{-1} was prepared. One hundred microliters of each solution was mixed with 1.0 mL of the col-

loidal gold preparation (D_{520} = 1.0). The mixtures were incubated for 10 min at room temperature with stirring. Then 0.1 mL of 10% NaCl solution was added to each preparation and D_{580} was measured after 10 min stirring at room temperature.

2.9. Conjugation of anti-CAP antibodies with colloidal gold

The antibodies were dialyzed against 10 mM sodium carbonate buffer, pH 8.5. Potassium carbonate solution (0.1 M) was added to the colloidal gold solution (D_{520} = 1.0) until it reached pH 8.5, and then the antibodies were added using the ratio of reactants chosen based on the analysis described above. The mixture was incubated for 30 min with stirring at room temperature. BSA solution (10%) was added to a final concentration of 0.25%. The colloidal gold particles were separated from unbound molecules by centrifugation at 8000 × *g* for 30 min at 4 °C. The pellets were re-suspended in 50 mM potassium phosphate buffer, pH 7.4, containing 0.1 M NaCl and 0.25% BSA. For long-term storage, 0.05% sodium azide was added to the conjugate.

2.10. Preparation of immunochromatographic test strips

Reagents were applied onto membranes using an IsoFlow dispenser (Imagene Technology, USA). Conjugate of colloidal gold with anti-CAP antibodies was dispensed from solution with D_{520} = 2.0; the conjugate load was 32 μL per 1 cm of strip width.

The analytical zone was formed by CAP–BSA or CAP–STI conjugate, the control area—by SAMIss (0.25 mg mL^{-1} in PBS, containing 10% (v/v) of glycerol in both cases). Two microliters of CAP–protein conjugates and SAMIss was applied per 1 cm of strip width.

After assembling membrane components, the cards were cut into strips of 3.5 mm width by an Index Cutter-1 (A-Point Technologies, USA). Length of test strips was 75 mm. Each test strip was packaged in laminated aluminum foil by FR-900 mini-conveyor (Wenzhou Dingli Packing Machinery Co., China), using prepackaged silica gel (0.5 g) as desiccant. Splitting and packing were carried out at 20–22 °C in a special room with relative humidity under 30%.

2.11. Sample preparation

Milk with the fat content of 0.5–4.2%, kefir with the fat content of 3.2% and 1%, and drinkable yogurts were purchased in a supermarket. Samples of these products were supplemented with different amounts of CAP and mixed thoroughly. Milk samples were analyzed in immunochromatographic assays directly, whereas kefir and yogurt samples were diluted with water (1.5 mL of sample + 0.5 mL of water).

2.12. Immunochromatographic assay and data processing

The assay was carried out at room temperature. The proposed test strip was vertically submerged into a sample for 2.5–3 min, removed and placed on a horizontal surface. After 10 min the assay results were recorded.

Binding of the colloidal gold particles at control and analytical zones was registered using a Bear Paw 4800TA pro scanner (Mustek, Taiwan) followed by digital processing of the images or using a Reflekom photometric device (Octa-Medica, Russia) [31,32]. Brightness value of 1.0 relative unit, as registered by the Reflekom, corresponds to the limit of reliable visual detection of the lines.

3. Results and discussion

3.1. Obtaining and characterization of the test system compounds

The anti-CAP antibody was characterized preliminary in the ELISA format of immunoassay. As demonstrated in Fig. 1, the working range of CAP detection by the competitive ELISA technique

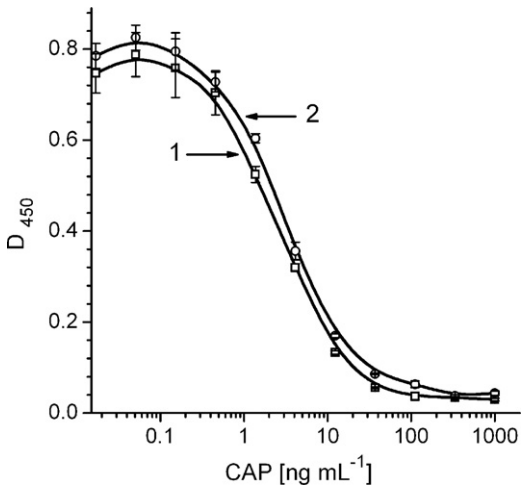

Fig. 1. Calibration curve of CAP ELISA using CAP–BSA (1) and CAP–STI (2) conjugates. The ELISA was carried out as described in Section 2.

corresponds well to concentrations relevant for control of dairy products (IC10 is 0.4 ng mL^{-1} for CAP–BSA and 0.5 ng mL^{-1} for CAP–STI). Cross-reactivity of the G35222 M antibody with other antibiotics was extremely low, less then 0.001% for gentamicin, streptomycin, amoxicillin, ampicillin, penicillins G and V (data not shown). Both coating conjugates, CAP–BSA and CAP–STI, had similar characteristics in ELISA and both were utilized in the immunochromatographic studies.

The principle behind the test (Fig. 2) is based on the competition between CAP in the sample and immobilized CAP–protein conjugate for binding to anti-CAP antibodies labeled by colloidal gold. Intensity of a colored line in the zone with the immobilized protein–CAP conjugate is reversely proportional to the amount of CAP in the sample.

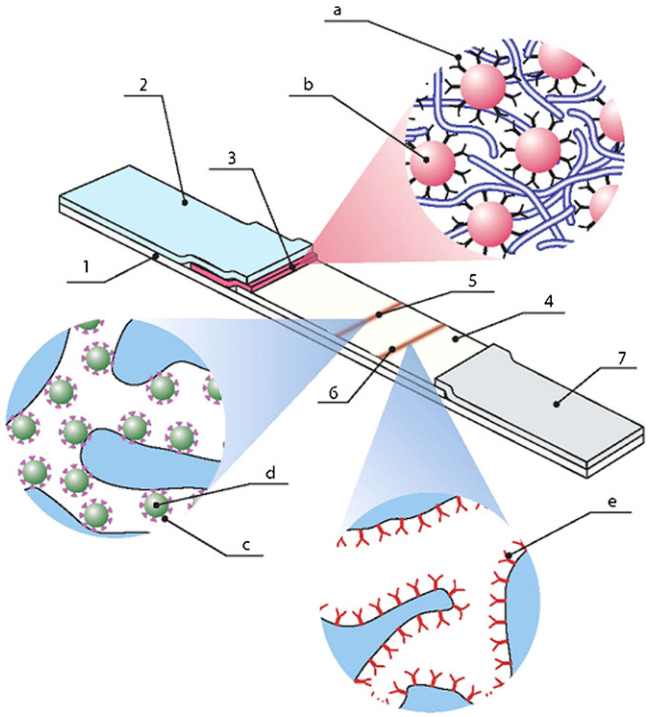

Fig. 2. Composition of immunochromatographic tests: (1) polyester backing, (2) sample pad, (3) conjugate release matrix (a—anti-CAP antibodies, b—colloidal gold particles), (4) nitrocellulose membrane, (5) analytical zone (c—CAP, d—protein, BSA or STI), (6) control zone (e—anti-mouse antibodies), (7) adsorbent pad.

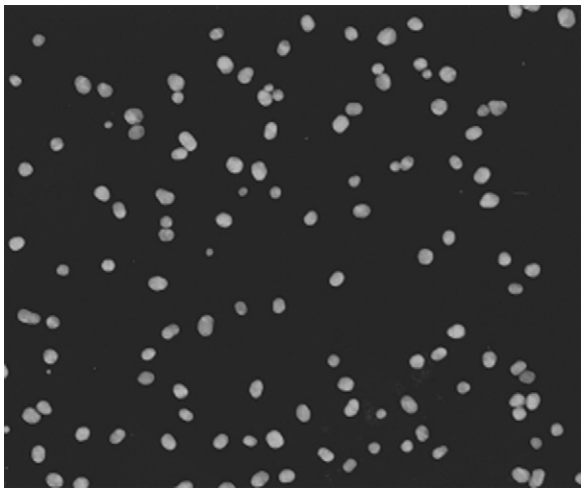

Fig. 3. Electronic microphotography of the colloidal gold preparation. Images were obtained using a CX-100 electron microscope under accelerating voltage 80,000 V and enlargement 3,300,000.

Preparations of colloidal gold were obtained and conjugated with anti-CAP antibodies as recommended by Chandler et al. [33]. Colloidal gold particles were obtained under conditions that allow them to reach an average diameter of 30–40 nm. Fig. 3 presets the data of electron microscopy of the particles. Data for 90 identified particles (after exclusion of overlaps) were processed. The average value of major axis of particles was 37.2 ± 7.9 nm, minor axis—29.6 ± 5.2 nm, i.e. particles were relatively uniform in size. The degree of ellipticity of the particles was 1.25 ± 0.15, reflecting that the shape of particles was close to spherical.

The concentration of antibodies required for conjugation with colloidal gold particles was determined based on dependence of optical density at 580 nm from antibody concentration (so called adsorption isotherm). When increasing the amount of antibodies, D_{580} increases, reaches a maximum, and starts to decline, reaching the plateau that corresponds to saturation of the particles by immobilized antibodies (Fig. 4). According to common recommendations [30], concentration of antibodies equal to 12 $\mu g\,mL^{-1}$ was chosen for the conjugation. This concentration results in antibody:colloidal particle molar ratio of 312:1 during synthesis.

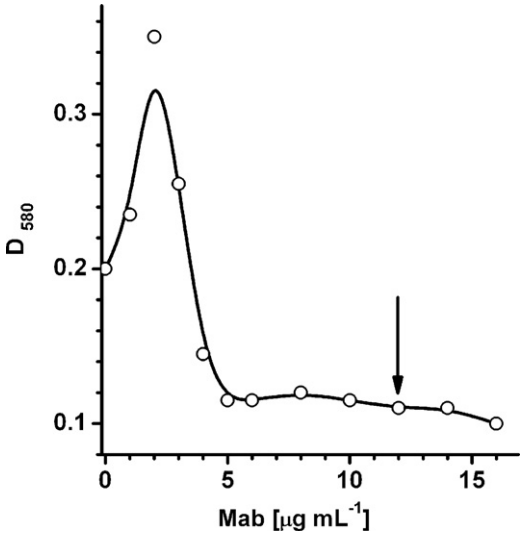

Fig. 4. Concentration dependence of colloidal gold optical density at 580 nm after adding different concentrations of anti-CAP antibody for the choice of the optimal concentration for conjugation.

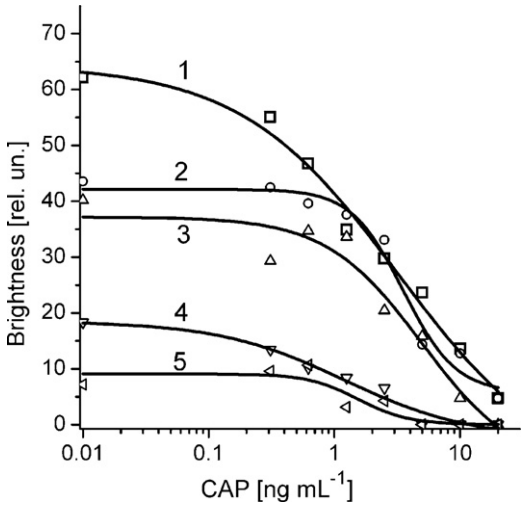

Fig. 5. Choice of CAP–STI dosage for analytical zone of the test strip. CAP–STI concentrations were 1, 0.5, 0.25, 0.12 and 0.06 mg mL^{-1} for curves 1–5 correspondingly. The conjugate of colloidal gold with antibodies was applied from the concentration that corresponded to $D_{580} = 2.0$. The profiles were registered after 5 min of the assay.

Selection of membrane compounds of the test strip was carried out according to Millipore recommendations on optimal conditions for immunochromatography of different kinds of samples. Test strips included a nitrocellulose membrane CNPF (average pore size 10 μm), conjugate release matrix PT-R7, a sample pad GFB-R7L, an absorbent pad AP045 and a cover tape MT.

3.2. Development and optimization of the immunochromatography protocol

At the initial stage of development, the ability of the synthesized antibody–colloidal gold conjugate to bind with specific reagents at the analytical and control zones of the test strip was tested. The testing results were used to select optimal preparations of these reagents for immobilization.

A series of polyclonal (goat, rabbit and sheep) antibody preparations raised against mouse immunoglobulin G were compared as potential agents for binding the colloidal conjugate at the control zone. At saturating concentrations of the anti-mouse antibodies, brightness of the formed line was equal to 35 relative units for the preparation from goat, 19.5 units—from rabbit, and 45—from sheep (data not shown). Therefore, we selected the sheep antibodies for further use in the analysis.

Coloration of analytical zone is different for immobilization of CAP–STI and CAP–BSA conjugates. The CAP–BSA conjugate provides a fuzzy analytical zone, whereas the CAP–STI conjugate—a zone with clearly defined edges. This observation led to the choice of the CAP–STI conjugate for immobilization.

The concentration of the CAP–STI conjugate in analytical zone was varied from 0.06 to 1 mg mL^{-1} (Fig. 5). The concentration of 0.25 mg mL^{-1} was optimal, as it provided for the desired sensitivity (10 ng mL^{-1} according to MRL), sufficient brightness of the analytical zone, and low consumption of the reagent.

The optimal signal was registered for the colloidal gold–antibody conjugate solution with the D_{520} of 2.0, which provided a satisfactory brightness of the control zone and no membrane background staining.

3.3. Characterization of analytical properties of the assay

A series of test strips was manufactured based on the optimized parameters of the proposed immunochromatographic system. Effi-

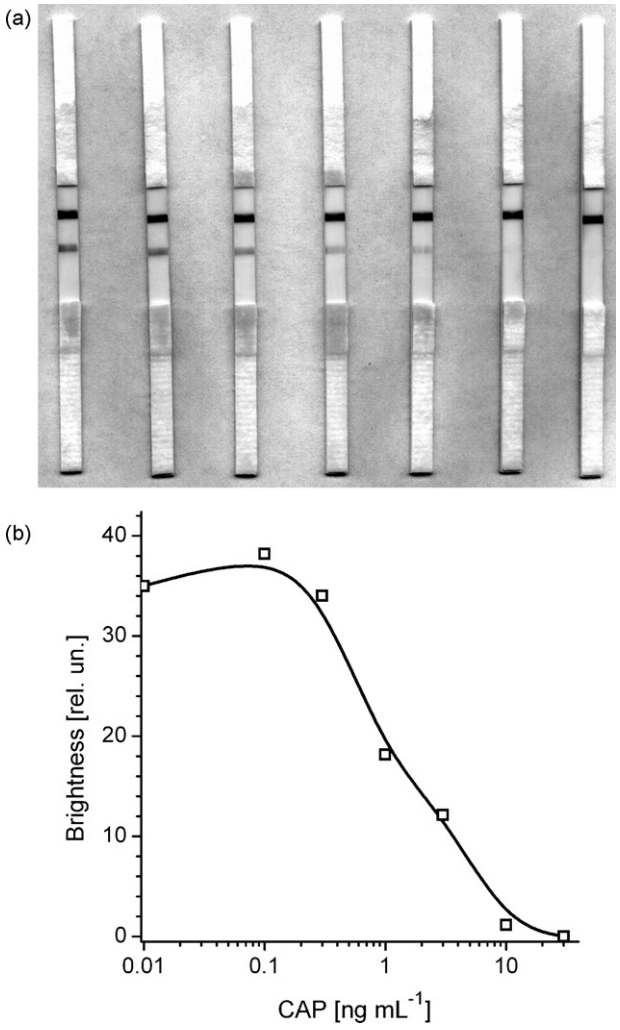

Fig. 6. Detection of chloramphenicol in PBST. (a) Test strips, (b) calibration curve. CAP concentrations in the samples (from left to right): 0, 0.1, 0.3, 1, 3, 10 and 30 ng mL^{-1}.

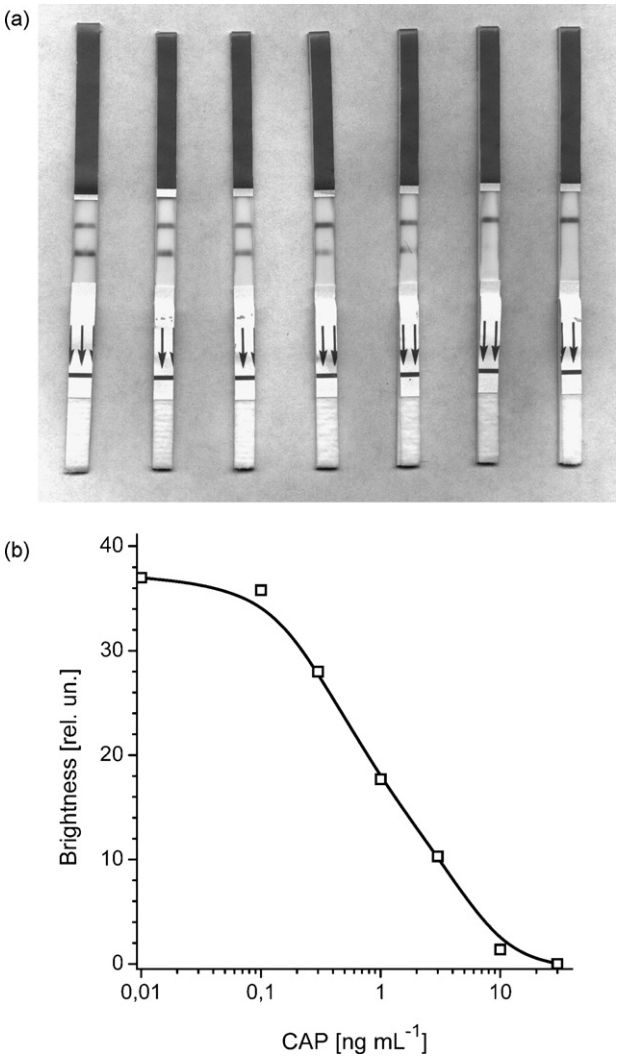

Fig. 7. Detection of chloramphenicol in milk (3.5% fat). (a) Test strips, (b) calibration curve. CAP concentrations in the samples (from left to right): 0, 0.1, 0.3, 1, 3, 10 and 30 ng mL^{-1}.

ciency of chloramphenicol detection in milk and buffer solutions was compared. Figs. 6 and 7 present the results of analysis of samples with different concentrations of chloramphenicol in PBST and in milk with 3.5% fat content.

At the conditions of the assay its cut-off level (point of (dis)appearance of the line in the analytical zone) corresponds to the MRL CAP value of 10 ng mL^{-1}. The assay duration is 10 min. In contrast to other available membrane tests for antibiotics, analysis described here can be carried out at room temperature. The results of the assay can be controlled visually or documented using a reflectometric device, which is based on a digital camera with the appropriate software.

The test systems were applied for analysis of milk, kefir and drinkable yogurts. For the all samples tested, possibility of reliable detection of chloramphenicol with the same cut-off level has been confirmed.

For comparison with existing analytical techniques milk samples with 1.5% and 3.5% fat content and different concentrations of CAP were tested using the manufactured test strips and commercial "RIDASCREEN® Chloramphenicol" ELISA kits. It should be noted that the application of immunochromatographic test for qualitative assay (exceeding the controlled threshold level) gives possibility only to compare assay data in terms of presence or absence of the controlled antigen. In this respect full conformity between conclusions about CAP content (as compared with MRL value of 10 ng mL^{-1}) was demonstrated for all 28 milk samples that were tested both by immunochromatography and ELISA. Considering the immunochromatographic assay with quantitative registration of the brightness in the analytical zone, it is possible to estimate correlation between its results and determination of CAP content by reference ELISA technique. Our measurements with scanner use have shown that for milk samples with 3.5% fat content and CAP content in a range from 0.1 to 10 ng mL^{-1} the correlation coefficient of the assays results was equal to 0.932 (n = 10), and for milk samples with 1.5% fat content and the same CAP content the correlation coefficient was equal to 0.960 (n = 10). These data testifies to suitability of the developed method for a quantitative estimation of CAP content in dairy production.

4. Conclusions

The intensive and not always justified use of antibiotics in veterinary medicine and their accumulation in foodstuffs lead to risks to human health. Here we describe development of a system for rapid detection of chloramphenicol. The application of immunochromatographic technique will allow to realize rapid (10 min) assay with deposition of complete set of reactants onto test strip, direct

induction of specific interaction by strip's contact with sample to be tested and possibility of visual detection of label (colloidal gold) binding in defined zones of the test strip. Since no sample treatment and heating is required unlike described earlier immunochromatographic techniques, the assays can be carried out directly at places of sampling. The assay is labor-consuming one and can be carried out without any additional devices and reactants. Due to their speed and simplicity, the assay developed here can become efficient tool for quality monitoring in dairy industry.

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References

[1] M.L. Bennish, Adv. Pediatr. Infect. Dis. 14 (1999) 269–290.
[2] S. Schwarz, C. Kehrenberg, T.R. Walsh, Int. J. Antimicrob. Agents 17 (2001) 431–437.
[3] B. Hardy, Anim. Biotechnol. 13 (2002) 129–147.
[4] C. Ibrahim, Int. J. Med. Microbiol. 296 (2006) 51–54.
[5] EC Commission Decision 2002/657/EC of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.
[6] EC Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of maximum residual permisable limits (MRPLs) for certain residues in food of animal origin.
[7] New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards (2008) Amendment No. 1.
[8] SanPiN 2.3.2.560-96, Hygienic Regularities for the Quality and Safety of Food Raw Materials and Food Products, 1997, Moscow, Russia (in Russian).
[9] Russian Federal Law N 88-FZ, Technical Requirements for Milk and Dairy Products, June 12, 2008 (in Russian).
[10] L. Lynas, D. Currie, C.T. Elliott, D.G. John, J.D.G. McEvoy, S.A. Hewitt, Analyst 123 (1998) 2773–2777.
[11] V. Gaudin, N. Cadieu, P. Maris, Food Agric. Immunol. 15 (2003) 143–157.
[12] L.K. Sorensen, T.H. Elbaek, H. Hansen, J. AOAC Int. 86 (2003) 703–706.
[13] P.A. Guy, D. Royer, P. Mottier, E. Gremaud, A. Perisset, R.H. Stadler, J. Chromatogr. A 1054 (2004) 365–371.
[14] L. Penney, A. Smith, B. Coates, A. Wijewickreme, J. AOAC Int. 88 (2005) 645–653.
[15] H.T. Ronning, K. Einarsen, T.N. Asp, J. Chromatogr. A 1118 (2006) 226–233.
[16] J.O. Wesongah, G.A. Murilla, A.N. Guatai, C. Elliot, T. Fodey, A. Cannavan, J. Vet. Pharm. Ther. 30 (2007) 68–73.
[17] L. Rodziewicz, I. Zawadzka, Talanta 75 (2008) 846–850.
[18] A.Y. Kolosova, J.V. Samsonova, A.M. Egorov, Food Agric. Immunol. 12 (2000) 115–125.
[19] M. Pattarawarapan, S. Nangola, C. Ayapiwatana, Chiang Mai J. Sci. 33 (2006) 85–94.
[20] X. Chuanlai, P. Cifang, H. Kai, J. Zhengyu, W. Wukang, Czech J. Food Sci. 23 (2005) 251–256.
[21] S. Lin, S. Han, Y. Liu, W. Xu, G. Guan, Anal. Bioanal. Chem. 382 (2005) 1250–1255.
[22] V. Gaudin, P. Maris, Food Agric. Immunol. 13 (2001) 77–86.
[23] I.S. Park, N. Kim, Anal. Chim. Acta 578 (2006) 19–24.
[24] Z. Peng, Y. Bang-Ce, J. Agric. Food Chem. 54 (2006) 6978–6983.
[25] C.A. Marquette, L.J. Blum, Biosens. Bioelectron. 21 (2006) 1424–1433.
[26] G.A. Posthuma-Trumpie, J. Korf, A. van Amerongen, Anal. Bioanal. Chem. 393 (2009) 569–582.
[27] J.W. Jang, Y. Jin, M.H. Lee, Korean J. Vet. Pub. Health 30 (2006) 247.
[28] K. Li, L. Liu, C. Xu, X. Chu, Anal. Sci. 23 (2007) 1281–1284.
[29] T. Fodey, G. Murilla, A. Cannavan, C. Elliott, Anal. Chim. Acta 529 (2007) 51–57.
[30] G.T. Hermanson, Bioconjugate Techniques, Academic Press, London, UK, 1996.
[31] B.B. Dzantiev, A.V. Zherdev, V.O. Popov, Y.Y. Vengerov, T.A. Starovoytova, R.T. Toguzov, Clin. Lab. Diagn. 8 (2002) 25–31 (in Russian).
[32] N.A. Byzova, Y.A. Balandina, A.V. Zherdev, B.B. Dzantiev, Allergy Asthma Clin. Immunol. 7 (2003) 189–192 (in Russian).
[33] J. Chandler, T. Gurmin, N. Robinson, IVD Technology Magazine 6 (N 2) (2000), http://www.devicelink.com/ivdt/archive/00/03/004.html.