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Development of a Competitive Indirect ELISA for the Determination of Lincomycin in Milk, Eggs, and Honey

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Polyclonal antibodies to lincomycin (LIN) were developed in rabbit as a result of immunization with BSA−LIN conjugate. Periodate oxidizing of hapten was the common step of both immunogen synthesis and preparation of conjugated antigens for coating plates (homologous and heterologous). Several ELISA variants on a base of the different antigens immobilized on polystyrene were compared. Heterology of solid-phase antigens was provided with relative hapten clindamycin (CLIN) and ethylene- or hexanediamine as spacer arm between hapten and carrier. The spacer insertion yielded no desirable effect, whereas gelatin−CLIN assay variant showed better test characteristics in comparison with the homologous one, although insignificant (IC_{50} was 9.15 vs 18.3 ng mL^{-1}). The detection limits of the developed test, being estimated as 0.43 ng mL^{-1} (milk) and 0.65 ng mL^{-1} (eggs), were sufficient to measure maximum residue levels for LIN in examined matrices. This value for honey was 1.9 ng mL^{-1} (1.3 μg kg^{-1}). The assay sensitivity was enough to dilute milk, egg, and honey samples by 10−100 times to minimize matrix effect. The examination of matrix effect and simple ways of its overcoming are detailed in the paper. The developed assay showed 111% cross-reactivity with CLIN; therefore, it is suitable for the determination of both lincosamides.

KEYWORDS: Lincomycin residues; immunoassay; milk; eggs; honey

INTRODUCTION

Lincomycin (LIN) is an antibiotic derived from *Streptomyces lincolnensis*. It belongs to the lincosamide group, which also includes pirlimycin and clindamycin. Lincomycin is active mainly against Gram-positive bacteria. It acts as an RNA-dependent inhibitor of protein synthesis. In veterinary medicine, it is administered with the feed or drinking water to poultry and as intramuscular or intramammary injections to dairy cattle. In human therapy, LIN has been largely replaced by clindamycin.

LIN does not possess prominent toxicity (*1*, *2*); however, its residues in foodstuff as well as other antibacterials may provoke microbial resistance generation. This leads to lack of efficiency in its therapeutic usage. That is why the limitation of LIN content in foodstuff is established in the European Union (EU) as maximum residual levels (MRL) for all producing species: 100 μg kg^{-1} (muscle), 50 μg kg^{-1} (fat), 500 μg kg^{-1} (liver), 1500 μg kg^{-1} (kidney), 150 μg kg^{-1} (milk), and 50 μg kg^{-1} (eggs) (*3*). The norms set by the Codex Alimentarius Commission are the same except those for fat (100 μg kg^{-1}) and muscle (200 μg kg^{-1}) (*4*). This antibiotic is effective for foulbrood disease treatment (*5*), and its residues may contaminate honey. Nevertheless, no MRLs have been established for antibiotics in bee products, although some countries, for example, Switzerland, the United Kingdom, and Belgium, have set limits, which generally lie between 10 and 50 μg kg^{-1} for each antibiotic group (*6*).

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Numerous available methods for the determination LIN in different matrices are generally based on chromatographic techniques with various detection systems (*7*−*10*). Immunoassay as a screening test has an advantage over chromatographic procedures in simplicity, sensitivity, and capability of high sample throughput. Immunochemical methods of determination have been developed for many antibacterials (*11*, *12*, *16*−*23*). However, an immunoassay of LIN has not been described yet.

The present investigation is the first trial of production antibodies against LIN and development of enzyme-linked immunosorbent assay (ELISA) for the determination of LIN in food matrices such as milk, eggs, and honey.

MATERIALS AND METHODS

Chemicals. Hydrochloride of LIN and CLIN phosphate, human transferrin (TF), *N*-hydroxysuccinimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *o*-phenylenediamine, and skim milk were purchased from Fluka-Sigma-Aldrich (Moscow, Russia). Glutaraldehyde and sucrose were from Serva (Heidelberg, Germany). Peroxidase-conjugated donkey antibodies to rabbit IgG, bovine serum albumin (BSA), and gelatin (gel) were obtained from Pharmateh (Moscow, Russia). Milk and eggs were from regional supermarkets. Honey samples were kindly provided by the All-Russian Research Institute for Veterinary Sanitation, Hygiene and Ecology.

Equipment. The microtiter plates were incubated in a plate thermoshaker, ST-3 L (ELMI Ltd. laboratory equipment, Riga, Latvia). The absorbance was read using a microtiter plate reader (model MR 5000 from Dynatech Laboratories, Denkendorf, Germany).

Conjugated Antigen Synthesis. *BSA−LIN(pi), Gel−LIN(pi).* Two solutions containing 12.6 mg of LIN (31 μmol) and 6.7 mg of $NaIO_4$

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(31.3 μmol) in 1 mL of water were combined and stirred for 20 min. Portions of 242 and 645 μL of this mixture (75- and 200-fold molar excess of hapten over protein carrier) were added to BSA solutions (7 mg, 0.1 μmol) in 0.05 M carbonate buffer, pH 9.5 (CB). The volumes of periodate-oxidazed LIN in 8, 24, and 80 μL (10-, 30-, and 100-fold molar excess) were poured in solutions of gel (4 mg mL^{-1}, 0.025 μmol). After 2 h of stirring at room temperature, the mixtures were supplemented with 0.1 mL of $NaBH_4$ (2 mg mL^{-1}) and incubated for another 2 h. The unreacted ingredients were removed by dialysis against distilled water.

BSA−EDA−LIN(ga), Gel−EDA−LIN(ga). Two microliters of 1,2-ethylenediamine (EDA, 30 μmol) was injected into the solution in the same way as oxidized LIN (29 μmol). Following 2 h of stirring, 0.1 mL of $NaBH_4$ (2 mg mL^{-1}) was added, and the mixture was kept until the next day. Water solutions of 7 mg of BSA (0.1 μmol) in 1 mL were combined with 176 and 469 μL (75- and 200-fold molar excess, respectively) of the resulting product that included EDA-modified LIN (EDA-LIN). The portions in 5.9 and 29 μL (10- and 50-fold molar excess, respectively) were added to solutions containing 4 mg of gel (0.025 μmol). These premixes were supplemented with 30 μL of freshly prepared 2.5% glutaraldehyde solution and mixed for 2 h using a magnet stirrer. For the next 2 h the mixtures were exposed to $NaBH_4$ (0.1 mL of 2 mg mL^{-1} solution) and then dialyzed against water overnight.

TF−HDA−LIN(pi). Six milligrams of crystalline $NaIO_4$ was dissolved in 2 mL of a water solution of 8 mg of transferrin (TF, 2 × 52 nmol) with 20 min of stirring and then were removed in the course of dialysis against 0.01 M acetate buffer, pH 5.0, during the night.

According to the above procedure LIN was also oxidized with sodium periodate, and the resulting solution (450 μL, 13.9 μmol) supplemented with 1.63 mL (10 mg mL^{-1}) of 1,6-hexanediamine (HDA, 14 μmol) was stirred for 2 h. Then 0.1 mL of $NaBH_4$ (2 mg mL^{-1}) was poured and left overnight at 4 °C.

The portions corresponding to 3.9 μmol (519 μL) and 10 μmol (1330 μL) of HDA−LIN were mixed with half a volume of oxidized TF (52 nmol) and incubated for 2 h with stirring. Molar ratios of HDA-modified hapten and protein carrier were 1:75 and 1:200. After reaction, the product was reduced with sodium borohydride (0.1 mL of 2 mg mL^{-1} solution), and the exhausting dialysis was carried out.

Gel−CLIN(pi) and Gel−EDA-CLIN(ga). The conjugated antigens based on clindamycin (CLIN) were synthesized like LIN conjugates, keeping the molar ratios of hapten to carrier as 1:10 and 1:50.

All of the conjugates were supplemented with an equal volume of glycerol and stored as 1 mg mL^{-1} solutions at −20 °C.

Immunization Procedure. Rabbits were immunized subcutaneously with 100 μg of BSA−LIN × 200(pi) at several points on the back. At the first administration the immunogen was emulsified in complete Freund's adjuvant; subsequent injections were carried out monthly with water solutions of this conjugate. For immune response monitoring 1 week after each booster immunization, a portion of blood was taken from the rabbits' ear marginal vein; serum was separated, supplemented with an equal volume of glycerol, and stored at −20 °C until testing.

ELISA Optimization. An optimal immunoreagent ratio was judged by the results of antigen−antibody cross-titration in indirect ELISA. The 96-well plates were coated with conjugated antigens in 0.2 mL of CB during the night at 4 °C. The concentrations of antigens were in range of 0.05−1.5 μg mL^{-1}. The plates were washed three times with 0.15 M phosphate-buffered saline, pH 7.0, containing 0.05% Tween 20 (PBS-T) and then were filled with 0.1 mL of PBS-T and 0.1 mL of antiserum serially diluted in the same buffer supplemented with 1% BSA. After incubation for 1 h in a humid chamber of a thermoshaker at (25 °C, 300 rpm) and a subsequent washing step, the solution of peroxidase-conjugated antibodies to rabbit IgG were added in 0.2 mL into wells and kept for an hour at 37 °C. Unbound material was removed with washing, and substrate mixture (0.4 mg mL^{-1} *o*-phenylenediamine and 0.005% hydrogen peroxide in 0.15 M citrate−phosphate buffer, pH 5.0) was poured. The enzymatic reaction lasted for 45 min and then was stopped with 50 μL of 4 M sulfuric acid per well. The colored product of reaction was measured using a photometer at 492 nm.

The ratios of immunoreagents (antiserum dilutions and concentrations of solid-phase antigens) that provided a reaction optical density in the range of 0.8−1.2 were determined and then used in competitive indirect ELISA.

Competitive Indirect ELISA. The conditions of the competitive assay procedure were the same as described above. The 96-well plates were coated with conjugates in optimal concentration. After washing, the standards of antibiotic (1000−0.1, 0 ng mL^{-1}) (B, B_0) or samples in 0.1 mL were added to each well together with 0.1 mL of antiserum in optimal dilution in PBS-T with 1% BSA. The plates were left at 25 °C for 1 h and then washed again; the wells were filled with 0.2 mL of a solution of anti-rabbit IgG antibodies conjugated with horseradish peroxidase. After 1 h of incubation and washing, the wells were filled with 0.2 mL of the substrate mixture and the assay was finished as described above.

For each concentration of antibiotic standard (n = 5) or spiked sample (n = 3) an antibody binding rate was calculated as B/B_0 and expressed in percent. The dependence of these indices was demonstrated as a standard curve plotted using OriginPro 8.0 software. The detection limit of the assay (LOD) for each matrix was evaluated as $B_0 - 3 \times$ standard deviation (SD). The cross-reacting of antibodies was assessed as the percentage of LIN concentration that induced 50% inhibition of antibody binding with antigen (IC_{50}) to the corresponding concentration of CLIN or another antibiotic.

Matrix Effect Examination and Sample Preparation. The matrix effect is an undesirable nonspecific hindrance of sample ingredients on antibody−antigen binding that results mostly in optical signal decreasing. It puts obstacles in the correct measurement of analyte in sample. The simplest way to overcome such a problem is to diminish this effect by diluting and/or masking the effect with the same or similar matrix medium. The degree of matrix effect and its dependence on sample dilution were examined and compared with antibody binding rate in PBS-T.

The pretreatment of milk samples meant only their dilution in PBS-T. The egg contents (egg white and yolk) were thoroughly mixed until the formation of a homogeneous liquid. This mélange was diluted with PBS-T, too, and analyzed in ELISA. Honey samples were melted in a warm water bath (50 °C). Then a 1 mL portion of dense syrup was taken in an insulin syringe without a needle and added to 1 mL of water for solubilization. The mean weight of 1 mL portions of different honeys was found as 1.4 g. Half-diluted honey samples were added to equal volumes of 86% water/acetonitrile (AcCN), stirred vigorously, and then centrifuged for 5 min at 1000 rpm (50*g*) for precipitate isolation. The liquid phase of the sample was taken, diluted in PBS-T, and used for testing.

Spikes Experiments and Recovery Determination. The LIN-free samples of milk, mélange, and honey were spiked with LIN to make 1000, 100, and 10 ng mL^{-1} final concentrations. The fortified samples were kept for a day at room temperature with occasional stirring, prepared by necessary mode, and operated in competitive ELISA. The percentage correspondence between spiked and found LIN concentration represented the drug recovery rate.

RESULTS AND DISCUSSION

Conjugated Antigen Synthesis. The principle of synthesis for all of the conjugates was identical. It was based on periodate oxidation of hydroxyl groups in the pyranoside fragment of lincosamides to aldehydes, which were able to react with amine-containing substances with Schiff base formation and following stabilization with sodium borohydride. To avoid excessive cleavage of the carbohydrate fragment of the molecule, an equimolar ratio between sodium periodate and hapten was chosen. The supposed structures of conjugates are shown in **Figure 1**.

Many works have demonstrated that structural heterology of coating antigen in comparison with immunogen played an essential role for improvement of assay sensitivity (*11*−*14*). Such differences meant not only another hapten carrier to remove anti-carrier antibodies reaction but perhaps also provided a heterologous relative hapten, different linking method, and insertion of a spacer arm between the hapten and protein carrier. So far as the conjugation was the same for immunogen and coating antigens, the desirable heterology of the last ones was realized, owing to spacers and alternative hapten. Insertion of a spacer such as EDA or HDA between the protein and hapten provided heterologous disposition of hapten more distantly from the carrier in comparison

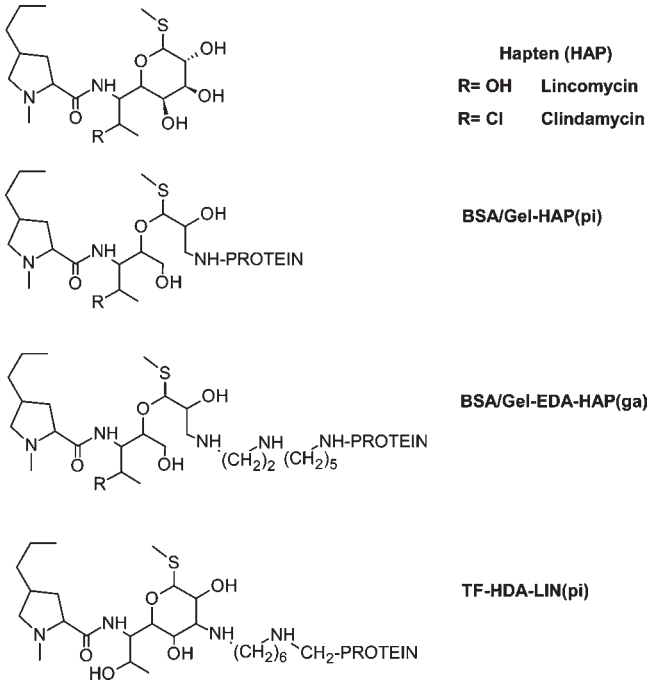

Figure 1. Structures of haptens and hapten−protein conjugates.

with immunogen. Being used for the synthesis of coating conjugates, CLIN−hapten served as an additional heterologous factor. Haptens and spacers were taken in equimolar ratios to provide complete reagents consumption and to form derivatives (HAP−EDA or HAP−HDA) with minimal quantity of unreacted compounds. There was a reason to avoid isolation and purification of intermediate product. The possible admixtures of unmodified hapten and spacer were either nonreactive in subsequent conjugation step or had no dramatic effect on immunochemical properties of conjugates.

Because both LIN and CLIN had no significant absorption in the UV−vis range from 230 to 360 nm (*15*), it was impossible to confirm the conjugate formation spectrophotometrically. Prepared conjugates were judged for their immunochemical properties, that is, binding with antibodies. Of the panel of conjugates differently loaded with hapten, the antigens that provided the best assay sensitivity were selected.

Antisera Testing, ELISA Optimization, and Cross-Reactivity Studies. BSA−LIN × 200(pi), an antigen with maximum hapten/protein molar ratio (200:1) by synthesis, was chosen for immunization. The first antiserum obtained after the first reimmunization (the second immunogen administration) showed its capability to bind with every conjugated antigen as well as to recognize free hapten that inhibited such binding in a dose-dependent manner. The solid-phase antigens with minimal hapten/carrier ratio by synthesis, gel−LIN × 10(pi), gel−EDA−LIN × 10(ga), and gel−CLIN × 10(pi), promoted better ELISA sensitivity than the others.

The next booster injection with antigen in initial dose (100 μg) resulted in the assay with sensitivity 5−11 times higher, which reached 9.15 ng mL^{-1} (IC_{50}) using gel−CLIN × 10(pi) for coating plates (**Figure 2**). However, the prolongation of the same dose immunogen administration contributed to worse ELISA parameters. The suspicion of tolerance provoked by high hapten density and large repeated doses of immunogen forced us to decrease the quantity of BSA−LIN × 200(pi) to 70 μg. The moderate positive effect recorded (serum N4) was observed no longer after the subsequent immunization course. At the same time, the antiserum titer became lower. Comparative examination of three

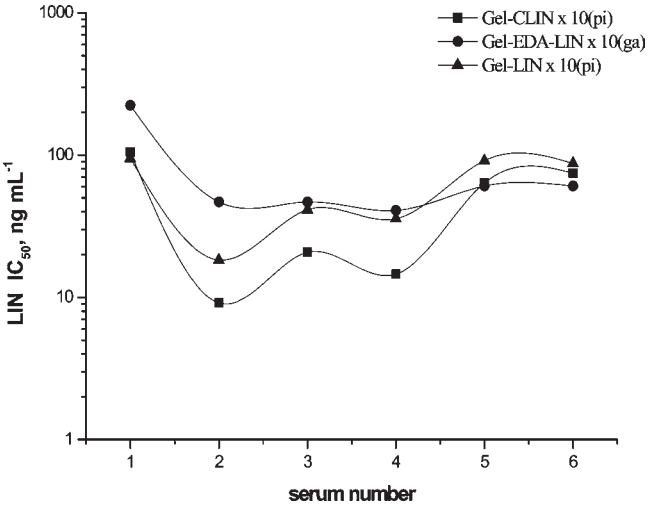

Figure 2. Immune response against LIN during immunization course; comparison of ELISA variants based on immobilized gel−CLIN × 10(pi), gel−EDA−LIN × 10(ga), and gel−LIN × 10(pi) and antiserum samples. The assay sensitivity is expressed in IC_{50} values calculated on standard curves for each pair of immunoreagents. Each point represents the average of three well replicates.

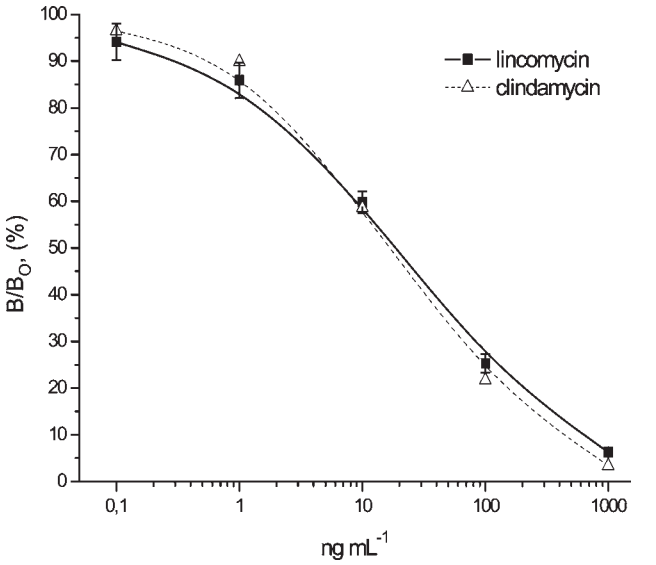

Figure 3. Standard curve of competitive indirect ELISA for determination of lincomycin and clindamycin. Each point represents the average of five replicates, and error bars represent standard deviations.

coating conjugates, the homologous gel−LIN × 10(pi), heterologous in spacer gel−EDA−LIN × 10(ga), and heterologous in hapten gel−CLIN × 10(pi), revealed a moderate advantage of the last one. Therefore, antiserum N2 and immobilized gel−CLIN × 10(pi) provided the best sensitivity of ci-ELISA. The assay variant using this pair of immunoreagents was selected for the following experiments (**Figure 3**).

Cross-reactivity of CLIN was defined as the percentage of LIN concentration that induced half-inhibition antibody binding to corresponding CLIN concentration, IC_{50} LIN/IC_{50} CLIN. This index was 111% for CLIN. Gentamicin, kanamycin, streptomycin, apramycin, tetracycline, penicillin, and chloramphenicol showed no detectable inhibiting activity at the 1 μg mL^{-1} level. Thus, the developed test showed specificity to lincosamide antibiotics. A group-specific assay with almost equal recognition of LIN (100%) and CLIN (111%) appears to be selective in practical

detection of the veterinary drug LIN and of CLIN used mainly in human medicine.

Matrix Effect Examination, Sample Preparation, and Recovery Estimation. *Milk*. Ingredients composing milk had a quite prominent effect on binding antibody with antigen. This was manifested in optical signal decrease even when the sample was diluted 100 times. To imitate such interference we used a reagent "skim milk". When prepared according to the manufacturer's instruction, the reagent solution showed a similar effect as normal milk matrix. This reagent solution diluted in PBS-T by a factor of 10 or 100 like samples was a medium for preparing LIN standards. Although the optical density level of reaction in PBS-T differed from that in the imitator, the corresponding standard curves were almost identical (**Figure 4**). The detection limit of LIN assay for milk testing was found to be 0.43 ng mL^{-1}. The recovery results from samples spiked with LIN in a wide range of concentrations and diluted 10 and 100 times are presented in **Table 1**. The measurement range was shown to be 1000−10 and 10000−100 ng mL^{-1} depending on 1:10 or 1:100 sample dilution, respectively. Nevertheless, the assay sensitivity was sufficient to reveal a MRL for LIN (150 ng mL^{-1}) in both situations with recovery rates of 82−115%. However, the contamination level of 10 ng mL^{-1} can be detected if the sample is diluted 10 times. In the case of 100-fold dilution, the same content of antibiotic was lower than the assay LOD.

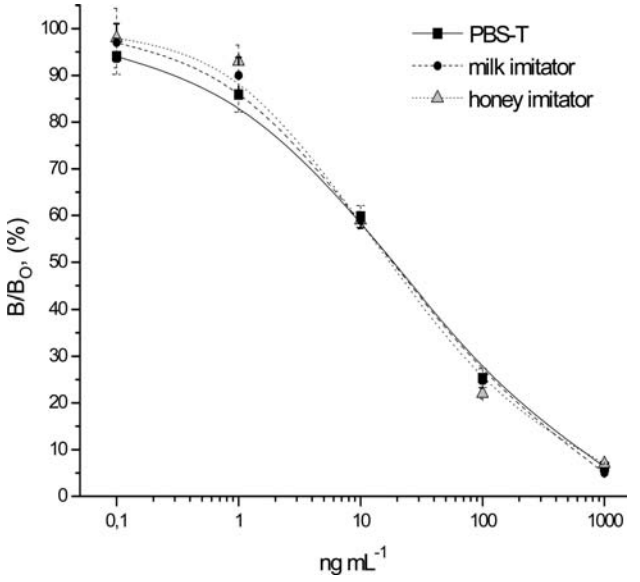

Figure 4. Standard curves for determination of lincomycin in matrices using the developed competitive indirect ELISA. The imitator of the milk matrix is a solution of skim milk diluted 1:10 with PBS-T. The honey imitator is PBS-T with 10% sucrose and 10% AcCN. These solutions and PBS-T were used as media to prepare LIN standards while testing milk, honey and eggs, respectively. Each point represents the average of five replicates, and error bars represent standard deviations.

To ascertain the thermal influence of sterilization or pasteurization on LIN stability, several samples with known drug concentration were incubated in a boiling water bath and then tested in ELISA. The exposure for 5, 10, and 15 min had no effect on drug quantification; that is, LIN immunochemical activity was not changed. Therefore, antibiotic degradation during the treatment of raw milk did not occur, and immunoassay determination of LIN was possible before thermal treatment of milk as well as after it.

A panel of milk samples (n = 106) was wide enough for testing the developed assay. It included the various kinds of milk purchased during 2008−2009 in supermarkets of different European regions of the Russian Federation. This examination permitted the degree of antibiotic contamination to be revealed. The concentration of LIN appeared to be > 150 μg kg^{-1} in two cases (224 and 260 ng mL^{-1}) (*16*).

Eggs. Egg sampling consisted of a homogenization procedure or, in other words, the preparation of a mélange. In comparison with other objects, the matrix effect of such a blend was minimal. It required only dilution of the mélange in PBS-T. Owing to the assay dynamic range (**Figure 4**) it was possible to determine a LIN MRL for eggs (50 μg kg^{-1}) and concentrations both an order above and an order below this level. Although the estimated detection limit of the assay ($B_0 - 3 \times$ SD) was 0.65 ng mL^{-1}, the experimental data demonstrated a wholly satisfactory recovery rate (108%) by 100-fold dilution of samples spiked with 10 ng mL^{-1} (**Table 1**). Besides, this 100-fold dilution was preferable to obtain the better values of recovery (90−108%). The procedure of sampling for LIN detection was simple and required no organic solvent extraction stage in contrast to egg pretreatment described for the analysis of sulfachlorpyridazine, fluoroquinolones, and nitroimidazoles (*17*−*19*).

Honey. Honey is a complex product including carbohydrates, vitamins, proteins, enzymes, organic acids, microelements, impurities sch as pollen, etc., which may be drawbacks for determination. The initial titration of several samples of white, flower, and buckwheat honey in PBS-T showed its strongly pronounced matrix effect (**Figure 5**). This inhibiting influence on reaction antibody−antigen occurred even with 100-fold sample dilution and reached on average an 11% decrease in optical signal. At the same time the degree of this effect varied widely depending on honey sort.

Because honey consists of 75−80% of carbohydrates (fructose, glucose, sucrose, and other saccharides), the concentrations of which may be different, the influence of these components on immunologic reaction was also examined. These experiments showed the matrix effect of honey was in part due to its carbohydrate component. Among tested carbohydrates an inhibiting activity of sucrose was higher than that for monosugars, glucose and fructose (**Figure 5**). The similar influence of both

Table 1. Recovery of Lincomycin from Artificially Contaminated Foodstuffs

		recovery (%) for samples spiked at concentrations		
matrix	sample dilution	1000 ng mL^{-1}	100 ng mL^{-1}	10 ng mL^{-1}
milk	1:10	115 ± 6.8[a]	105 ± 6.8	104 ± 5.4
	1:100	82 ± 11.1	85 ± 5.7	170 ± 11.5
eggs	1:10	77.1 ± 23.6	84.0 ± 13.8	117.6 ± 68
	1:100	99.9 ± 37.2	90.4 ± 9.9	108 ± 27
white honey	1:20	95 ± 3.5	91.3 ± 2.9	92.7 ± 0.9
flower honey	1:20	97.8 ± 14.7	94.2 ± 11.5	110 ± 8.0
buckwheat honey	1:20	112.7 ± 8.0	103.7 ± 16.5	136 ± 46.3

[a] Standard deviation samples of foodstuffs (n = 3).

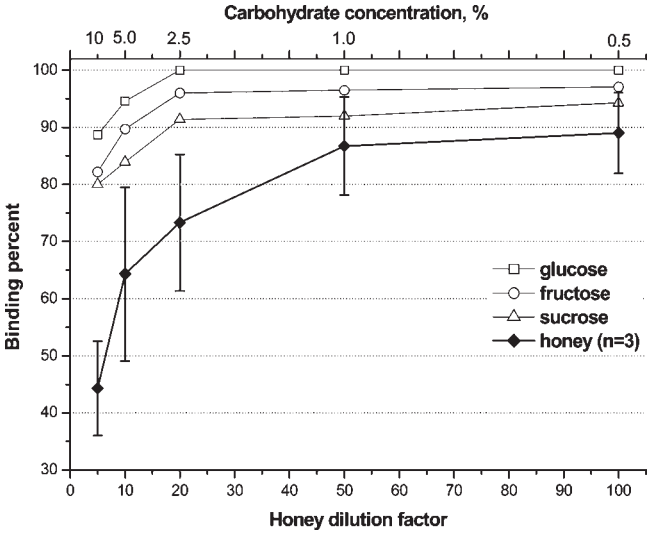

Figure 5. Effect of honey and carbohydrates on antibody binding rate. Honey and sugars were titrated in PBS-T.

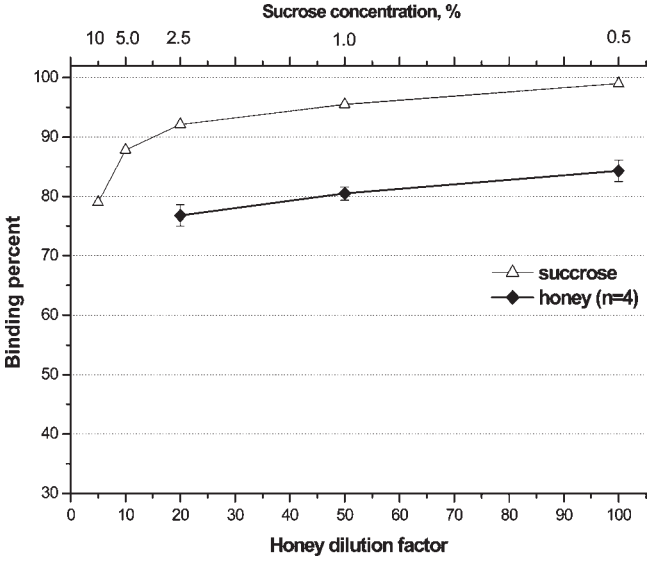

Figure 6. Effect of honey and sucrose on antibody binding rate. Honey and sugar were titrated in PBS-T containing 10% AcCN.

strong sugar and honey solutions was registered in ELISA tests specific to aminoglycoside antibiotics, tetracyclines, chloramphenicol, fluoroquinolones, bacitracin, and tylosin (data not shown). Thus, the observed effect was not associated with the features of immunoreagents applied.

Honey filtration through a 0.22 μm membrane that aimed evidently at removal of pollen or other impurities (*20*) had no desirable effect. By analogy with work (*21*) to avoid possible metal ion effects, the dilution of honey in buffer with 0.05 M EDTA (pH 7.0) was used. That medium appeared to intensify the honey matrix effect.

Treatment of half-diluted honey with an equal volume of AcCN, a 5-fold dilution of liquid phase with PBS-T, and following titration in 10% AcCN−PBS-T buffer was carried out to estimate signal level. For comparison, **Figure 6** presents the titration curve of sucrose as a sugar with substantial inhibitory effect. The medium of titration, PBS-T containing 10% AcCN, served as B_0. Although the degree of honey drawbacks was not reduced in comparison with PBS-T-extraction, the individual sort differences ($n = 4$) were almost eliminated. The evidence of this fact is the small error of measurement (SD) (**Figure 6**). The level of antibody binding in 10% sucrose solution (79%) being almost the same as the inhibition activity of a 1:20 honey solution (77%) provoked the use of sucrose-containing buffer as a reagent that imitated honey matrix. Therefore, PBS-T with 10% sucrose and 10% AcCN served as a dilutor for the preparation of LIN standards. These conditions did not change the behavior of the standard curve critically; it was similar to that in PBS-T (**Figure 4**). The assay LOD was found to be 1.9 ng mL^{-1} (1.3 μg kg^{-1}). An efficacy of this approach was confirmed in a recovery test. The three most popular sorts of honey were spiked with LIN to make contamination levels of 1000, 100, and 10 ng mL^{-1} ($\times$0.7 μg kg^{-1}). The pretreatment procedure resulted in AcCN-induced precipitation of several ingredients. The precipitated material was localized intermediately between two liquid phases, the upper organic and the bottom syrup-like phases. After a short centrifugation step, these layers separated more clearly and material for testing could be taken from each liquid layer. Lincomycin residues were not detected in the organic phase, but the data of drug recovery from the bottom layer are shown in **Table 1**. Thus, the described procedure was proved to be a simple way to unify different honey sorts and reach an acceptable recovery rate (91−136%). Sucrose appeared to be a suitable reagent−imitator of honey matrix effect. It was a good alternative to honey composition that was applied for the same purpose in described immunoassays (*20*−*23*) and had to be preliminarily confirmed as antibiotic free.

Conclusions. This paper presents the first description of the preparation of antibodies and the development of an ELISA for the quantification of the lincosamide antibiotic lincomycin in food matrices. The cross-reactivity of clindamycin is equal to 111%, and so the developed test is suitable for the determination of both lincosamides and may be considered as a group-specific assay. Although the immunization scheme was not optimum, the assay appeared to be no less sensitive but was even more sensitive than the majority of described methods. The assay sensitivity allowed minimizing the matrix effect using 10- and 100-fold sample dilutions. Detection limits of 0.43 ng mL^{-1} (milk) and 0.65 ng mL^{-1} (eggs) were sufficient to measure the MRL of LIN in the examined matrices. Honey was preliminarily exposed to AcCN for deproteinization. Because of this pretreatment, the different sorts of honey were unified in matrix effect. To mask it, the sucrose solution was applied. By using this honey imitator it was possible to determine LIN up to 1.9 ng mL^{-1} (1.3 μg kg^{-1}). The data of spikes experiments showed satisfactory recoveries of 82−115% (milk), 77−118% (eggs), and 91−136 (honey) that proved the measurement using standard curves for LIN in matrix imitators was valid.

ABBREVIATIONS USED

BSA, bovine serum albumin; CB, carbonate buffer, pH 9.5; gel, gelatin; CLIN, clindamycin; EDA, 1,2-ethylenediamine; EDC, *N*-hydroxysuccinimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; ELISA, enzyme-linked immunosorbent assay; HDA, 1,6-hexanediamine; LIN, lincomycin; LOD, limit of detection; MRL, maximum residual level; TF, human transferrin; SD, standard deviation.

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