COMPARATIVE STUDY OF METHODS FOR THE DETERMINATION OF ANTIBIOTICS IN MILK AND DAIRY PRODUCTS

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Annotation: *In this work, a comparative study of methods for determining antibiotics in milk and dairy products, ensuring the uniformity of measurements and characteristics and measurement errors was carried out.*

Key words: *antibiotic, milk, delvotest.*

Russian standard and proprietary methods proposed by Russia for the determination of antibiotic residues in accordance with domestic and European standards, based on the standards ISJ 13969:2003 International Dairy Federation - 183 "Milk and dairy products". Established adequate solutions to the diagnostic level of the limits of detection of the maximum permissible concentration in raw milk.

The content of residual amounts of antibiotics in raw milk poses, on the one hand, a threat to the health of the consumer, and on the other hand, it is a technological risk in the production of dairy products. The control of antibiotic residues in raw milk is prescribed in the health legislation of most countries. European legislation is the strictest in this regard: for the vast majority of antibiotics actually used in animal husbandry, maximum permissible concentrations are established, and control procedures are standardized.

According to EU Directive 92/46, the procedure for setting maximum permissible concentrations must be carried out by “tested, scientifically sound methods, regulated, in particular, at EU or international level”. As regards arbitration methods, reference is made in the directive to Commission Decision No. 91/180, in which they are described in detail. Arbitration methods are divided into a qualitative method for the determination of antibiotics and methods for determining the presence and concentration of antibiotics of the penicillin group. The sensitivity requirements of the methods are also given.

As international standards in the EU, proprietary methods approved by AOAC international are recognized, in particular Delvotest (DSM, the Netherlands), SNAP (Idexx Inc., USA), Charm II (Charm Sciences Inc., USA), as well as some national standards, such as German LMBG standards for chromatographic methods for the determination of antibiotics in food products, including milk. The International Organization for Standardization and the International Dairy Federation, in turn, do not standardize a detailed procedure for the determination of antibiotics, but a description of the method, on the basis of which a reasonable conclusion can be drawn about the compliance of the method with normalized characteristics [1].

Russian SanPiN 2.3.2.1078-01 establishes the complete exclusion of residual amounts of penicillin, streptomycin, chloramphenicol and tetracycline group antibiotics in raw milk, indicating in the note the concentration, obviously, as the required detection limit for the methods used. As methods of analysis, SanPiN 2.3.2.1078-01 allows “metrologically certified methods that meet the requirements for ensuring the uniformity of measurements and the characteristics of measurement

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errors, methods of using product samples in testing and controlling their parameters, as well as methods that meet the specified requirements and approved in the prescribed manner, without giving direct references to the documents establishing the procedure for certification and approval. On the other hand, the reference appendix to the SanPiN contains several guidelines for the determination of residual amounts of antibiotics in food products, including milk, and GOST 23454-79 on methods for determining inhibitors in milk (indicated in GOST R 52054-2003 "Natural milk cow-raw materials. Technical conditions"). In addition, there is GOST 51600-2000 “Milk. Method for the determination of antibiotics”, approximately corresponding to the EU arbitration method. In general, the Russian nomination of the content of residual amounts of antibiotics and methods of their control is similar to the European one, but it is written much less clearly and completely[2].

The processes of Russia's integration into the world economy objectively require the harmonization of approaches to product quality assessment. The new technical regulatory framework that is emerging in our country, in particular the Technical Regulations for Milk and Dairy Products, is largely oriented towards European legislation. The purpose of our study was to evaluate Russian standard and proposed Russian branded methods of determination in terms of their adequacy to responsible and European standards based on ISO 13969 (MMF 183) Milk and dairy products. Guidelines for Standards for the Description of Bacterial Growth Inhibition Tests, ISO 18330: (MMF 188) “Milk and dairy products. Guidelines for a standardized description of immunological bacterial receptor methods for the determination of antimicrobial residues” [3].

Materials and methods

As milk free from inhibitors, we used raw milk from one of the suburban farms of the Almaty region, tested for the absence of inhibitors according to GOST-23454-79, antibiotics according to GOST 51600-2000, and t as well as the Charm II method.

For the study, in addition to the title antibiotics of the group (penicillin and tetracycline), the most widely used in the treatment of mastitis amoxicillin, cloxacillin, chlorriooxytetracyclic at concentrations from 4 to 0.25 MPC EU were selected.

Dilutions of antibiotic standards penicillin, tetracycline (manufactured in Russia) and commercial preparations of amoxicillin, cloxacillin, oxytetracycline, chlortetracycline (Krka, Slovenia) were introduced into milk. Dilutions were prepared according to the method MU3049-84 "Methodological guidelines for the determination of residual amounts of antibiotics in animal products" and successively diluted to working concentrations of 0.016; 0.008; 0.004; 0.002; 0.1;0.05; 0.025; 0.012; 0.006 μg/cm3 (u/g) – cloxacillin; 0.5; 0.25; 0.12; 0.06; 0.03 μg/cm3 (u/g) - for antibiotics of the tetracycline group. In addition, when determining the number of positive results, the SKIV preparation (according to GOST 23454-79) manufactured by Biocompass LLC was used as a negative control along with raw native milk.

Each concentration of all antibiotics and negative controls were determined in five replicates by each test method. The study used the following methods and a test kit for determining residual amounts of antibiotics: MU 3049-84 "Guidelines for the determination of residual amounts of antibiotics in animal products" (hereinafter MU-84): MUK 4.2.026-95 "Express - method for determining antibiotics in food."

• GOST 51600 - 2000 "Milk. Methods for the determination of antibiotics". Cup method with Bacillus stearothermophilus (hereinafter Bac.stear.);

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• GOST 51600-2000. Bromocresol purple indicator method (hereinafter Delvotest);

• Draft Amendment No. 1 to GOST 51600-2000. Method with 3,3,5,5-trimethylbenzidine and dimethol sulfoxide (hereinafter SNAP);

• Test kit "Blue-Yellow" manufactured by Charm Sciences Inc., USA (hereinafter Charm BY);

• Test kit "Charm MRL" manufactured by Charm Sciences Inc., USA;

• Charm II test kit manufactured by Charm Sciences Inc., USA.

MUK-95, Bac.stear., Delvotest and Charm BY are qualitative methods based on the inhibition of bacterial growth and do not distinguish between the type of inhibitor. MU-84, also based on inhibition of bacterial growth, is a quantitative method for determining the type of antibiotic. SNAP, Charm MRL and Charm II are immune (bacterial) receptor antibiotic-specific methods, of some SNAP and Charm MRL are qualitative, and Charm II is a semi-quantitative method.

Since five of the eight methods studied are braking tests, the ISO 13969:2003 standard (MMF 183) was taken as the basis for reporting the results.

The following indicators were determined:

1. Limit of detection with a probability of 95% (DL 95%) - the concentration of the antibiotic at which this method gives 95% of positive results. Doubtful results were always regarded as negative. 95% OL was determined by a graphical method: points on the graph showing the percentage of positive results (Y-axis) at a given antibiotic concentration (X-axis) were connected by a line. The projection onto the X-axis of the point of intersection of this curve with the 95% line was considered the value of 95% OL.

2. The ratio of software 95% to MPC. This ratio shows how the method corresponds to the established MPCs: overly sensitive methods will give positive reactions at acceptable concentrations of TCA, insufficiently sensitive methods will not detect TCA at unacceptable concentrations.

3. Number (%) of false positives.

Results and discussion.

All methods, with the exception of MUK-95, demonstrated the linearity of the results and a direct dependence of the number of positive results on the concentration of the antibiotic. MUK-95 did not give either 100% positive or 100% negative results at any of the determined concentrations of any of the studied antibiotics, including negative controls. Therefore, MUK-95 was excluded from further study and thus the usefulness of antibiotic residues in raw milk is questioned. Figures 1 and 2 show graphs for the determination of 95% OR for amoxicillin and oxytetracycline, the table shows 95% OR for seven methods for six tested antibiotics.

Table 1.

Antibiotic, mcg/kg	**Limits of Antibiotics Detection by Investigated Methods**						
	MU-84	**Bac.stear.**	**SNAP**	**Charm MRL**	**Charm II**	**Delvotest**	**Charm BY**
Penicillin	6	7	3	3	1,7	3,5	3,7
Amoxicillin	7,4	6,8	6	3,5	1,6	6	6,8
Cloxacillin	94	96	42	24	22	45	48
Tetracycline	95	200	30	95	28	420	225
Oxytetracycline	95	225	30	95	28	420	230

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Chlortetracycline	90	185	30	94	28	215	175

Specific methods demonstrated the lowest detection limits: SNAP, Charm MRL and Charm II, with Charm MRL and Charm II being more sensitive to beta-lactam antibiotics, and tetracycline groups - SNAP and Charm II. Among the methods of inhibition of bacterial growth, the most sensitive to beta-lactams were Delvotest and Charm. BY, and tetracyclines - MU-84. It should be noted that none of the test methods corresponds to the required limits for the determination of tetracycline specified in SanPiN 2.3.2.1078-01 (10 times lower than the EU MPC).

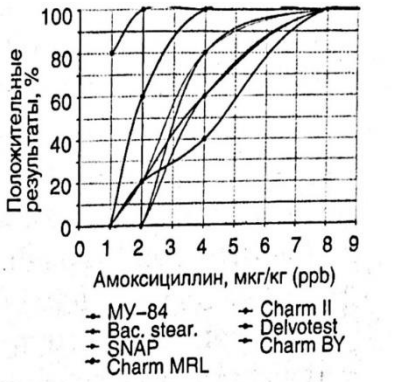

Рис. 1. График зависимости положительных результатов семи исследованных тестов от концентрации амоксициллина [1 мкг/кг (ppb)= = 0,001 мкг/см³ (ед/г)] в сыром молоке. Проекция точки пересечения графика с линией 95 % дает величину ПО 95 % (см. таблицу).

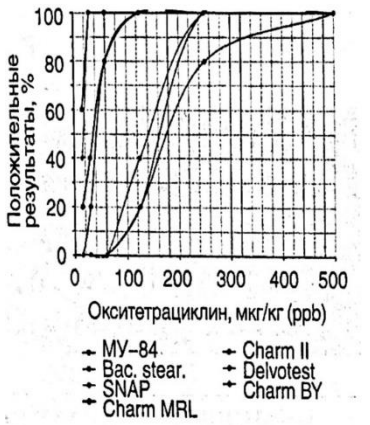

Рис. 2. График зависимости положительных результатов семи исследованных тестов от концентрации окситетрациклина [1 мкг/кг (ppb) = 0,001 мкг/см³ (ед/г)] в сыром молоке

Conclusion.

All the methods studied (with the exception of MUK-95) were able to reliably determine the presence of penicillin at the detection level required according to SanPiN2.3.2.1078-01, none of the methods was able to stably and reliably determine the presence of tetracyclines at the level specified in this document.

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