



THE SYSTEM OF MONITORING OF INHIBITORY DRUG RESIDUES IN RAW COWS' MILK IN SLOVAKIA

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ABSTRACT

This article provides brief information on the system of monitoring raw cows' milk for the presence of inhibitory veterinary drug residues in the Slovak Republic. We are describing in detail how the monitoring is carried out and what laboratory methods are used for this monitoring. We also deal with the issue of the disposal of contaminated milk. The presence of inhibitory veterinary drugs like antibiotics or residues of these drugs in milk in an amount exceeding maximal residual limits defined in legislation is illegal. Milk supplies containing detectable concentrations are not acceptable. The reputation of milk as a healthy and safe food should be protected. Dairy companies and consequently consumers want to be confident that milk and milk products are free of inhibitory veterinary drugs contamination. Small amounts of certain antimicrobial agents may affect antibiotic resistance in the human population, some percentage of the population is hypersensitive to antibiotics and other drugs. The presence of drug residues in milk affects the technological process of the production of fermented dairy products. Each country should implement a system of monitoring these drugs' residues in food. The system, which is applied in Slovakia, is effective, it can detect and the presence of these substances before milk processing in dairies. The total incidence of these substances in 2020 was 0.025% of all 12,181 samples tested in central testing laboratories. During the last 20 years.

Keywords: raw milk; drug residues; cow milk; antibiotics

INTRODUCTION

The system of monitoring of raw cow's milk for the presence of residues of inhibitory substances in Slovakia is performed in several stages of the food chain. This is a mandatory obligation arising from Commission Regulation (EC) No 1662/2006 (EC, 2006). For this Regulation, food business operators must initiate procedures to ensure that raw milk is not placed on the market if:

- it contains antibiotic residues in quantities which, in the case of any of the substances listed in Annexes I and III to Regulation (EEC) No. 2377/90 (EEC, 1990) exceeds the value permitted under that Regulation, or
- the combined total antibiotic residue level exceeds any maximum permitted level.

Regulation EEC No. 2377/90 (EEC, 1990) was repealed by regulation (EC) No. 470/2009 (EC, 2009a), but the annexes present in repealed regulation remain in force.

If raw milk does not meet these conditions, the food business operator must, following Commission Regulation (EC) No. 1662/2006 (EC, 2006) to notify the appropriate authority (Regional Veterinary and Food Administration of the Slovak Republic) and take measures to immediately solve this problem.

In the Slovak Republic, therefore, food business operators ensure compliance with the requirements of this Regulation through a multi-level control system.

The use of antibiotic therapy to treat and prevent udder infections in cows is a key component of mastitis control in Slovak Republic (Zajác, Golian, Vácziová, 2006; Zajác, Golian, Čapla, 2007a; Zajác, Golian Vácziová, 2007b; Kološta, 2007), Czech Republic (Navrátilová, 2008) and many other countries (Hillerton et al., 1999).

Factors to be considered to choose the most suitable method of residue detection are the type of antibiotic used, expected time limitations, sensitivity of the test, and costs (Senyk et al., 1990).

The antibiotic residue detection assay systems that are currently available use different methods and test organisms (Van Eenennaam et al., 1993).

Microbiological assays for the detection of antibiotic residues utilize the genus *Bacillus* sp. because of its high sensitivity to the majority of antibiotics. The *Bacillus stearothermophilus* var. *calidolactis* is routinely used by the dairy industry to screen antibiotic residues. Delvotest SP is a multiple microbial inhibitor test used to detect antimicrobial agents such as beta-lactams and sulpham compounds (Suhren and Beukers, 1998).

Microbiological tests Copan Milk Test and Delvotest SP-NT can be used for screening antimicrobial substances in milk (Le Breton, Savoy-Perroud and Diserens, 2007).

In general monitoring of antimicrobial agent residues, microbial growth inhibition methods, and rapid tests are used. Microbial inhibitor screening methods are easy to perform and enable the detection of a wide spectrum of agents. The microbial inhibitor screening methods do not attain with some antibiotics the sensitivity at the levels specified by the MRLs (maximum residue level). For the determination of these agents, other methods should be used (e.g. immunochemical or receptor). Another disadvantage is the long period needed to perform the test and the occurrence of falsely positive results if the tests are used for the analysis of individual samples containing higher levels of naturally occurring antimicrobial agents. Rapid tests enable to obtain the result of a test in several minutes and they are highly specific. A majority of the rapid assays were aimed above all, at the detection of β -lactam antibiotics. Even in these tests, falsely positive results can occur (Navrátilová, 2008).

The performance criteria described by the European Decision 2002/657/EC have to be used for validation of these tests (Le Breton, Savoy-Perroud and Diserens, 2007).

The quantification of these substances is performed by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (SVFI, 2021).

Scientific Hypothesis

The number of positive samples decreases during the observed period. On-farm milk testing with rapid veterinary drug residue screening tests by farmers or dairies is an effective tool to reduce the risk of processing milk contaminated with veterinary drugs.

MATERIAL AND METHODOLOGY

Samples

Raw cow milk samples were sampled on the farm by trained and certified personnel from dairy factories. These persons were trained by the Veterinary and Food Institute in Slovakia in National Reference Laboratory for Milk and Milk Products. The training course "Sampling of raw milk manually and by autosamplers for milk payment as part of self-inspection" is accredited by the Ministry of Education, Science, Research and Sport of The Slovak Republic (MESRS, 2020).

Chemicals

Samples were not preserved or preserved by Azidiol 0.02% (PanReac AppliChem).

Animals and Biological Material:

This research does not involve the use of animals or biological materials.

Instruments

Laboratories used water-baths 40 °C for sample preparation and incubators 64 °C \pm 0.5 °C and 65 \pm 0.5 °C for samples incubation.

Laboratory Methods

The following laboratory method was used:

- Delvotest[®] SP-NT (Supplier: Skar s.r.o. Bratislava, Slovakia);
- Eclipse 50 test (Supplier: Merck, Slovakia).

- CMT+ or formally Copan Milk test (Supplier: Climax, s.r.o., Bratislava);
- Twinsensor (Supplier: Merck Slovakia);

To determine residues of inhibitory substances, the instructions provided by the suppliers and requirements of the ISO/TS 26844 (ISO, 2006) were fulfilled.

In the Delvotest[®] SP-NT ampoules (Figure 1) with a culture medium containing *Bacillus stearothermophilus* var. *calidolactis* were used. The ampoules were incubated for 3 h at 64 \pm 0.5 °C and the color change was evaluated. Samples were considered as negative if the agar change the color from violet to yellow (MP, 2020; MARD, 2006).

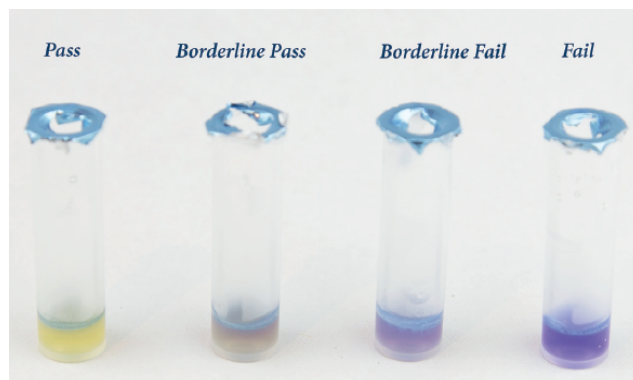


Figure 1 Reading results in Delvotest[®] SP-NT.

ECLIPSE 50 combines the principle of agar diffusion tests with a change in the color of the indicator due to the active metabolism of the test microorganism in the absence of an inhibitor. The Eclipse 50 kit has format microplates. The test sample is dispensed into a well-filled agar medium containing *Bacillus stearothermophilus* var. *calidolactis*. Incubation at 65 °C, at which normal growth of the test strain, causes the color of the pH indicator to change from blue to yellow. If they are under investigation in a sample of the growth inhibitory substance of the test strain, the color of the indicator remains blue-violet (MARD, 2009a).

Description of the Experiment

Sample preparation: samples were heated to 40 \pm 1 °C in the water bath and gently mixed.

Number of samples analyzed: The total number of analyzed raw cows' milk samples was 452,731.

Number of repeated analyses: Each sample was analyzed once. If a positive result was found, the analysis was repeated.

Laboratory samples were sampled manually according to the international standard ISO 707 (ISO, 2008) or by autosamplers according to the Slovak technical standard STN 57 0005 (STN, 2000) with calibrated autosampler.

Samples were stored at 5 °C and sent to one of two Central testing laboratories authorized by the Ministry of Agriculture and Rural Development of the Slovak Republic (MILEX Progres, a.s. Bratislava and EXAMINALA – Dairy Research Institute, Žilina).

Samples were analyzed by one of the above-mentioned laboratory methods. Results of the analysis were evaluated as positive or negative. Overall Results were sent to the Veterinary and Food Institute Bratislava, National reference laboratory for milk and milk products in Nitra, Slovakia to be processed and statistically evaluated.

Statistical Analysis

Firstly, the total number of analyzed samples per year was calculated. Secondly, the percentage of positive samples was calculated. These results were plotted on the graph (Figure 2). The reliability equation and the coefficient of R^2 were calculated by Microsoft Excell.

We have used ONE-WAY ANOVA to evaluate the effect of the rapid drug residues tests used by farmers. The results were statistically significant at a p-value less than 0.05.

The group of samples from 2001 was considered as a comparison group of samples because rapid tests were not yet widely used in Slovakia in this period.

The results of the group of samples from 2020 were analyzed against this comparison group of samples from 2001.

The statistical evaluation was performed by XLSTAT 2021.2 (Addinsoft).

RESULTS AND DISCUSSION

The results of the experiment are present in Table 1 and Figure 2. The overall number of analyzed samples per year decrease continuously from 34,795 (2001) to 12,181 (2020). This fact results from a change in legislation due to the accession of the Slovak Republic to the European Union, which took place on 1 May 2004, as well as from the subsequent decline in the number of dairy farms and the number of dairy cows. In 1989, 559 thousand dairy cows were bred in Slovakia and milk production reached more than two billion liters. At present, only less than a quarter of dairy farms are bred in Slovakia, and milk production has halved (Pravda 2019). The number of drug residues positive samples significantly ($p < 0.05$) decreases from 299 (2001) to 3 (2020). This is because, since about 2004, rapid tests to detect the presence of residues of inhibitory substances have become more widely used in Slovakia. Already in 2004, a significant decrease in positive samples to 114 was recorded. This trend continued in the following years and is related to the use of rapid tests. In 2019 and 2020, only 3 positive cases per year were recorded in central testing laboratories, which represents 0.025% of all tested samples. For comparison in 2001, the percentage of positive samples was 0.859%. However, we must note that these statistics are partly distorted because after the accession of the Slovak Republic to the EU the Regulation of the Government of the Slovak Republic No. 312/2003 (GOV SR, 2003) has been replaced by European legislation (EC, 2006) and the obligation to report positive samples by Regional Veterinary and Food Administrations to the National reference laboratory for milk and milk products has lapsed in Slovakia. These data are available in individual Regional Veterinary and Food Administrations but are not further statistically evaluated. Finally, our statistic contains only data reported by central testing laboratories and does not include positive cases reported directly by dairies to the Regional Veterinary and Food Administrations of the Slovak Republic.

In the following parts of the discussion, we describe in detail the system of monitoring these substances in Slovakia. From a theoretical point of view, this system takes place on several levels. Even based on strict control, we can state that the risk of the presence of residues of inhibitory substances in dairy products in Slovakia is minimal. Dairies only process milk without these substances.

Milk control system for the presence of inhibitory substances

The first level of control

The first stage of control is carried out directly on the farms using rapid screening tests carried out by the farmer or by the driver of the tanker truck before milk is pumped to the tanker and transported to the dairy. However, not all farmers or tanker truck drivers check freshly milked milk directly on the farm. From the dairy's point of view, this level of control directly at the farmer is interesting because it eliminates the problem of contamination of milk from other farmers in the truck, and also it eliminates the transfer of risk and potential problems with the presence of veterinary drug residues to the next stage of the food chain. Food business operators carry out this control for their own money to eliminate the risk of supplying milk containing antibiotic residues. The purchase of rapid screening tests is provided directly by farmers or by dairies and distributed to farmers or milk tankers drivers.

The second level of control

The second stage of control takes place at the entrance to the dairy as a self-verification check. Following EC Regulation No 852/2004 (EC, 2004a), the established HACCP system and monitoring for the presence of inhibitory substances are performed routinely during milk intake. Each dairy has a customized monitoring system. In most cases, the laboratory worker will firstly take samples from individual farms sampled by the truck driver or autosampler. Also, it is necessary to take the "tanker sample" which contains milk from all farmers. Usually, this sample is sampled automatically on farms if the autosampler is used. Milk from all farmers is collected in one big bottle. In the case of manual sampling, laboratory workers need to take the sample from the tanker cistern or each tanker cistern chamber. This can be made following ISO 707 (ISO, 2008). Sometimes this tanker sample is sampled by dripping milk from the pipe valve leading to the receiving tank. Milk is waiting in the truck tanker or the designated intake tanker and can not be processed until is analyzed for drug residues presence. The sample is examined by a rapid 5 – 10 minute test (e.g. BetaStar, BetaStar Combo, ROSA Charm 3 SL3, ROSA Charm SLB, Twinsensor BT, SNAP BT ST test, etc.).

The sensitivity of rapid tests is either at the level of maximum residue limits or even higher (Zajác et al., 2006, 2007a, 2007b) and is suitable for milk testing by dairies.

If the residues of inhibitory substances are detected, then all samples from individual farms are tested. This is how the farm that caused the contamination is found. These positive samples are tested by the dairy only with a long 3-hour tests Delvotest[®] SP-NT (MARD SR, 2006), Eclipse 50 (MARD SR, 2009a), or Kalidos TB MP (MARD SR, 2009b). The dairy reports this situation to the appropriate Regional Veterinary and Food Administration, but as this is a self-verification check, these results are not statistically evaluated by this institution.

Some dairies do not perform a long 3-hours test but they send positive samples directly to the accredited laboratory for confirmation, or they automatically consider the milk to be contaminated and therefore intended only for disposal.

Table 1 Number of samples analyzed in central testing laboratories and number of drug residues positive samples in Slovakia.

Year	Number of analyzed samples	Number of negative samples	Number of positive samples	Percentage of positive samples
2001	34 795	34 496	299	0.859
2002	36 050	35 869	181	0.502
2003	33 402	33 210	192	0.575
2004	30 572	30 458	114	0.373
2005	30 324	30 250	74	0.244
2006	27 646	27 547	99	0.358
2007	26 024	25 992	32	0.123
2008	26 297	26 275	22	0.084
2009	23 313	23 286	27	0.116
2010	21 371	21 360	11	0.051
2011	19 475	19 458	17	0.087
2012	18 754	18 746	8	0.043
2013	17 965	17 959	6	0.033
2014	18 918	18 913	5	0.026
2015	17 440	17 436	4	0.023
2016	15 270	15 267	3	0.020
2017	15 321	15 317	4	0.026
2018	14 196	14 191	5	0.035
2019	13 417	13 414	3	0.022
2020	12 181	12 178	3	0.025

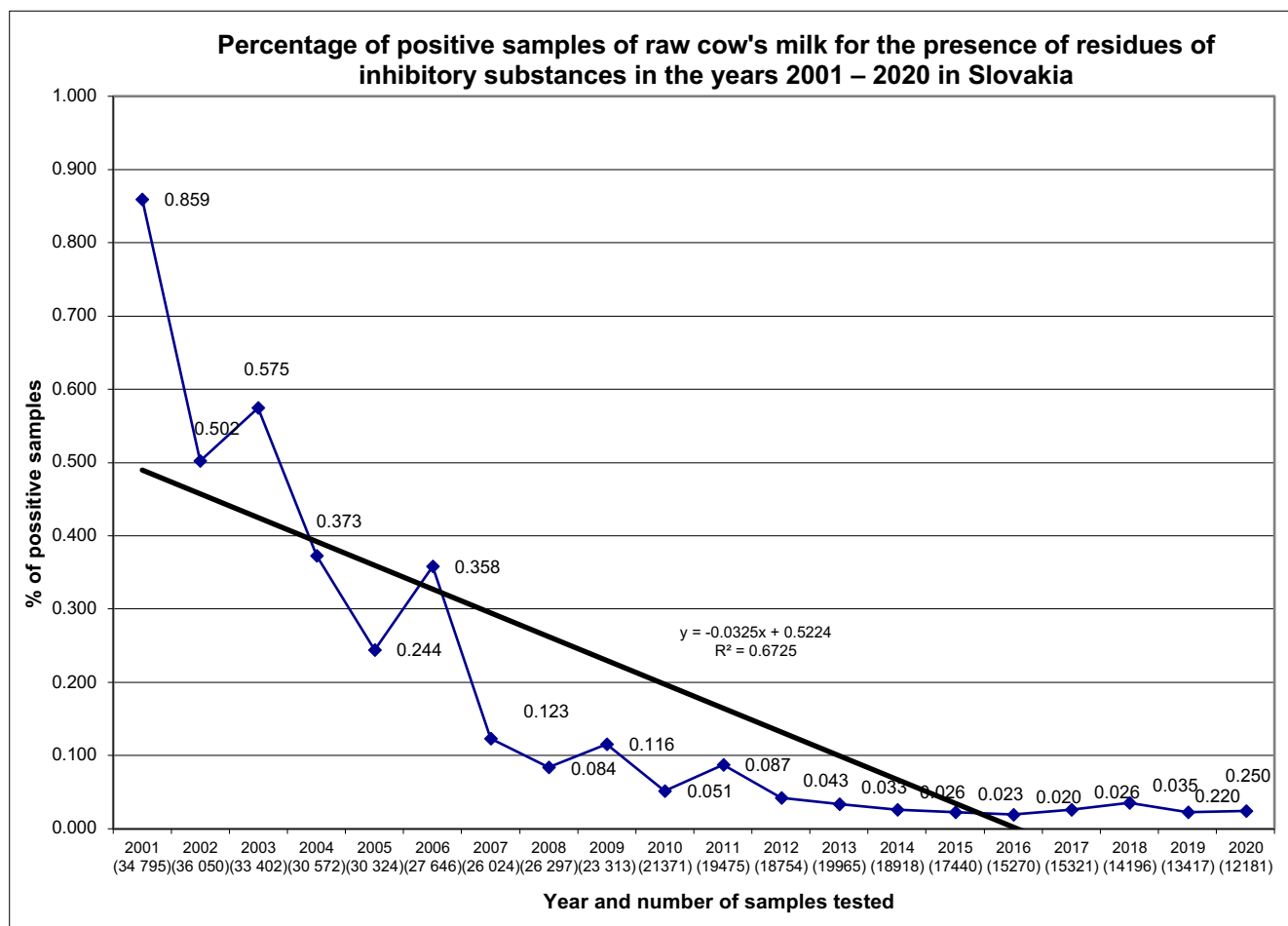


Figure 1 Percentage of positive samples of raw cow's milk for the presence of residues of inhibitory substances in the years 2001 – 2020 in Slovakia.

The damage must be paid by the farmer who caused such contamination. Usually, the incurred damage is resolved as an insured event.

In Slovakia or European Union, the legislation does not specify which laboratory should test samples (EC, 2006). Even the legislation does not require accreditation of this laboratory! In practice, this situation can be handled directly by the legislation of the country or by the supplier-customer contract. In Slovakia, it is usually tested in one of the two accredited central testing laboratories specifically intended for milk analysis or in the National Reference Laboratory for Milk and Milk Products in Lužianky or by the National Reference Laboratory for Antibacterial Substances and Other Pharmacologically Active Substances Dyes and Some Prohibited Substances in Dolný Kubín (MARD, 2020).

In practice, dairies usually use the services of accredited laboratories for confirmation of positive samples only rarely. They usually immediately consider the sample to be positive. This is how it is solved in most supplier-customer contracts. Confirmation of positive samples can be performed in a contracted accredited central testing laboratory or one of the above-mentioned national reference laboratories. When the accredited laboratory confirms the positivity of the sample, then milk is legally disposed of by the authorized company under a decision of the Regional Veterinary and Food Administration.

Some dairies in Slovakia have recently started sending milk samples for analysis of milk composition, total bacterial count, somatic cells count, and drug residues to laboratories in Hungary, Austria, Germany, or the Czech Republic. The results from these laboratories are then not statistically processed by National Reference Laboratory for Milk and Milk Products. Only the results from central testing laboratories in Slovakia are processed by NRL.

The dairy reports the presence of a positive case of residues of inhibitory substances to the Regional Veterinary and Food Administration, which will carry out an inspection at the farmer who caused the contamination.

After the correction, the farmer will request a Regional Veterinary and Food Administration for sampling the milk, which will then be examined in an accredited testing laboratory. Only after this analysis can the farmer deliver the milk to the dairy again.

The third level of control

The third stage of control is performed in central testing laboratories or any other laboratory. Dairies in Slovakia are usually sent milk samples for the inhibitory drug residues substances test to the contracted one central testing laboratory. The frequency of testing is not prescribed in European legislation (EC, 2006). Usually, two samples per month are collected from each farm. This testing is performed as a part of the verification process based on the HACCP plans. Also, the frequency of testing is mentioned in the supplier-purchaser contract. The testing is generally carried out in conjunction with the examination of the freezing point of the milk because it is recommended to analyze not preserved milk samples. On the other hand, for some tests, preserved samples may be used.

Persons taking milk samples for payment purposes, whether drivers or directly the purchaser's staff, must be trained by the Veterinary and Food Institute, National

Reference Laboratory for Milk and Milk Products. These persons periodically undergo an accredited educational activity every five years and hold a "milk sampler's certificate", which entitles them to take milk samples. In principle, such samples are taken in duplicate. One sample remains on the farm and the other is used by a dairy company. The sampling plan created by the dairy for payment must be secret. Farmers must not know the date of this sampling. Sampler notifies the farmer immediately when this sample is collected. All samples are sent to a contracted laboratory (usually a central testing laboratory).

If residues of inhibitory substances are detected in the second or third stage of control, the dairy shall trace the source of the contamination to identify the farm which caused the contamination.

The farm that caused the contamination will then provide financial compensation for milk and costs associated with the legal disposal of the contaminated milk to all farmers affected by this contamination. From a technical point of view, single-chamber or multi-chamber tanks can be used to transport milk. In most cases, the milk is contaminated in the relevant chamber of the entire milk tank into which the milk is pumped is contaminated. In some cases, contamination of several chambers in the milk tank may occur if the capacity of the currently filled chamber is insufficient or by cross-contamination from residues of contaminated milk left in the hoses and distribution system of the milk tank truck. In the case of using a multi-chamber tank, it is, therefore, possible to save part of the milk supply, since the positive milk is usually only found in one of the chambers of the tank.

In the case of confirmation of a "positive" result for the presence of antibiotic residues, the dairy plant shall carry out the following:

- does not accept (reject) the milk and sends it for disposal following the rules laid down in Regulation (EC) No. 1069/2009 (EC, 2009b) on animal by-products and derived products not intended for human consumption. The disposal of Category 3 material is carried out as follows:
- applied to land without processing,
- it can also be disposed of by composting or transformation into biogas.
- Such disposal is possible only in that case if the competent authority does not consider it a risk of any disease communicable through that product to humans or animals.

According to the Decree of the Ministry of Industry and Trade of the Slovak Republic No. 148/2012 (MIT SR, 2012), such disposal must be carried out only under the official supervision of a veterinary inspector with his consent. According to EC Regulation no. 142/2011 (EC, 2011), the removal of milk by the wastewater stream is prohibited.

Depending on how the buyer has stated in the supplier-customer contract with the farmer, he can ensure that another confirmatory test is performed, which identifies and quantifies the antibiotic and proves whether or not the maximum residue limit for this particular antibiotic is exceeded. These confirmation tests are usually performed in accredited testing laboratory: Veterinary and Food Institute Dolný Kubín, National Reference Laboratory for Antibacterial Substances and Other Pharmacologically

Active Substances, Dyes and Certain Prohibited Substances.

If the maximum residue limit is exceeded, the milk and milk products are disposed of legally. If the maximum residue limit is not exceeded, milk and products made from it are considered safe.

According to **Kološta (2007)**, the biggest problem is mainly residues of antibiotics and sulfonamides, as these are mostly used in the treatment of dairy cows. The reference method for the detection of antibiotics and sulphonamides in raw milk and heat-treated milk is the CD 91/180 / EEC method on the principle of microbial inhibition. The mentioned method was also taken over into the system of Slovak technical standards STN 57 0531 Determination of antibiotics and sulfonamides in raw and heat-treated milk (**STN, 2001**).

The journals of the Ministry of Agriculture and Rural Development of the Slovak Republic state which laboratory methods can be used for the official control of the presence of inhibitory substances in milk. These are methods based on the principle of agar diffusion tests with a change in the color of the indicator due to the active metabolism of the test microorganism (*Bacillus stearothermophilus* var. *Calidolactis*) in the absence of an inhibitor. The determination of residues of inhibitory substances in milk and milk products can therefore be carried out in central testing laboratories by the following methods approved by the Ministry of Agriculture and Rural Development of the Slovak Republic:

- Delvotest® SP-NT (**MARD SR, 2006**),
- Eclipse 50 (**MARD SR, 2009a**) or
- Kalidos TB MP (**MARD SR, 2009b**).

Also, there is a

- CMT+ test (**Climax, 2021**), which has been verified by the National Reference Laboratory for Milk and Milk products, but it is not listed in the list of approved methods of the Ministry of Agriculture and Rural Development of the Slovak Republic.

When testing raw cow's milk for payment, central testing laboratories in the Slovak Republic (MILEX Progres, as Bratislava and EXAMINALA – Dairy Research Institute in Žilina) use a diffusion test to determine residues of inhibitory substances, which lasts 3 hours: Delvotest® SP-NT. This test is used also by the National Reference Laboratory for Milk and Milk Products in Nitra (NRLM) (**Zajác and Čapla, 2010**).

However, it is not possible to determine the exact amount of antibiotic residues or a specific species using these microbiological or antigen methods.

Veterinary and Food Institute Dolný Kubín, National Reference Laboratory for Antibacterial Agents and Other Pharmacologically Active Substances, Dyes and Certain Prohibited Substances quantify veterinary drug residues by HPLC/MC method (**SVFI, 2021**).

The fourth degree of control

The State Veterinary and Food Administration of the Slovak Republic annually compiles the National Plan for the Control of Residues in Live Animals and Products of Animal Origin following the applicable legislation of the EU (**EC, 2017; EC, 2019**). The aim of the control through this plan is to obtain an overview of the current state of

contamination of individual components of the food chain, to implement effective measures to eliminate the identified deficiencies and prevent their further occurrence. Long-term monitoring of the presence of inhibitory substances in milk leads to the finding that almost 95% of cases of secondary contamination with inhibitory substances are antibiotic contamination, approximately 2.5% of contamination is due to other drugs, especially sulfonamides and residual detergent and disinfectant residues, 1% may cause mycotoxins from feed (nuclear, bulk), 1% may be caused by agrochemical residues or some preservatives and then only about 0.5% remains on natural inhibitors in milk (**Kološta, 2007**).

Commission Regulation (EC) No. 853/2004 (**EC, 2004b**) contains specific hygiene rules for food of animal origin.

Commission Regulation (EC) No 1662/2006 (**EC, 2006**) defines health requirements for the production of raw milk. Raw milk must come from animals to which no unauthorized substances have been administered and the products (medicines or substances) administered have complied with the withdrawal periods prescribed for those products. This Regulation also defines the requirements for milking, transport, and transport hygiene. According to the Regulation, it is necessary to identify animals that have undergone treatment which could cause the excretion of drug residues in milk and colostrum, and that milk and colostrum obtained from such animals should not be used for human consumption before the expiry of the prescribed withdrawal period. Dips or sprays pods will be used only after authorization or registration. Based on these requirements, primary milk producers must put in place systems to control the treatment of dairy cows.

In the past, the State Veterinary and Food Administration published a guideline for control of raw milk in farms for the production of raw milk (**SVFA, 2009**), today the control is based on the requirements of the current European legislation (**EC, 2017; EC, 2019**), Title IV, Article 49 Control of milk and colostrum production holdings and Article 50 Control of milk and colostrum.

The Regional Veterinary and Food Administration checks the food business operator whether, according to EC Regulation no. 1662/2006 (**EC, 2006**) has procedures in place to ensure that raw milk is not placed on the market if it contains antibiotic residues in quantities which, for any of the substances listed in Annexes I and III to Regulation (EEC) No. 2377/90 (**EC, 1990**) exceeds the value permitted under that Regulation or if the combined total antibiotic residue level exceeds any maximum permitted value.

During the official control, the inspector fills in the prescribed official control record, in which he states the number of reports of positive cases of residues of inhibitory substances in raw milk. It also checks whether the supplier and the relevant regional veterinary and food administration of the Slovak Republic have been informed about unsatisfactory results of raw milk. In case of unsatisfactory results of raw milk, the inspector checks the documentation, the date of detection (notification from the Central testing laboratory, the State Veterinary, and Food Institute, the own laboratory, or any laboratory), the date of written notification to the relevant Regional Veterinary and Food Administration and the raw milk supplier.

The food business operator must therefore take measures to remedy this situation and should not place milk

containing residues of inhibitory substances on the market. In the case of residues of inhibitory substances, the legislation does not further specify what measures the food business operator has to take. The legislation does not specify how the control body should proceed, nor does it specify whether samples are to be taken to prove remediation and where (what laboratory) these are to be tested. However, according to the methodological guideline (SVFA SR, 2009), inspectors of Regional Veterinary and Food Administrations are instructed as follows: the producer of raw milk must start procedures to ensure that raw milk is not placed on the market, i.e. unless corrective measures have been taken and it should be proved that raw milk does not exceed the maximum permitted level of the combined total antibiotic residues. Food business operators must not place such milk on the market. In practice, this means that after the situation has been remedied, the farmer must have a sample of milk examined at his own expense in the laboratory (it is not defined what kind of laboratory, but usually it is agreed with the inspector what laboratory should be used for this testing. Almost it is an accredited central testing laboratory or any accredited laboratory of the Veterinary and Food Institute – NRL for milk and milk products or NRL for Antibacterial Substances). If the result of the test shows that the milk does not contain antibiotic residues, it may place it on the market.

Control of antibiotic residues is necessary to protect the reputation of milk as safe food and to prevent exposure of the consumers to drug residues. Governmental authorities which are obliged to organize supervision and control systems by state supervision agencies must provide these requirements (Popelka, et al., 2003).

EU countries must monitor food of animal origin for the presence of residues. The legislation governing the design and implementation of residue monitoring plans is as follows (EC, 2021):

- Directive 96/23/EC - Annexes are in force and establish the sampling frequency and range of substances to be tested for.

- Decision 97/747/EC - Annexes are in force and establish additional sampling frequencies for milk.

- Regulation (EU) 2021/808 - Rules for the validation of analytical methods used in the residue monitoring plan and official sample treatment (Laboratory Analysis).

Specific EU legislation outlines the laboratory analysis and correct interpretation of results:

- Regulation (EU) 2021/808 establishes Criteria and procedures to validate analytical methods, ensuring the quality and comparability of the results of official laboratories. Common criteria for the interpretation of test results. Minimum required performance limits (MRPL) for analytical methods to detect substances without a maximum limit. This is relevant for substances that are not authorized or that are specifically prohibited in the EU. Also sampling procedures and official sample treatment.

Commission Decision 97/747/EC lays down that the annual number of samples taken should be one per 15,000 tonnes of annual milk production, with a minimum of 300 samples (EFSA, 2019).

The overview of the non-compliant results for prohibited substances is present in Table 2. Chloramphenicol is the most dominant prohibited substance detected in milk in European Union.

Table 2 Prohibited substances detected in milk in EU.

Year	Drug residue	Number of non-compliant results	Countries reporting non-compliant results	Source
2019	Chloramphenicol	3	Italy, Latvia, Poland	(EFSA, 2021)
2018	Chloramphenicol	2	Poland, Spain	(EFSA, 2020)
2017	Chloramphenicol	1	Croatia	(EFSA, 2019)
2017	AHD (1-aminohydantoin)	1	Croatia	(EFSA, 2019)
2017	AMAZ (5-methylmorpholino-3-amino-2-oxazolidone)	1	Croatia	(EFSA, 2019)
2017	AOZ (3-amino-2-oxazolidone)	1	Croatia	(EFSA, 2019)
2017	SEM (semicarbazide)	1	Croatia	(EFSA, 2019)
2016	Chloramphenicol	3	Poland	(EFSA, 2018)
2016	Hydroxymetronidazole (MNZOH)	1	Germany	(EFSA, 2018)
2015	Chloramphenicol	2	Latvia, Poland	(EFSA, 2017)
2014	Chloramphenicol	2	Latvia, Poland	(EFSA, 2016)
2013	Chloramphenicol	3	Latvia, Slovenia, Slovakia	(EFSA, 2015)
2012	Chloramphenicol	1	Spain	(EFSA, 2014)
2011	Chloramphenicol	1	Spain	(EFSA, 2013)
2010	Chloramphenicol	3	Czech Republic, Spain	(EFSA, 2012)
2009	Chloramphenicol	1	Slovakia	

Note: the list of the reports is present on the European Commission website (EC, 2021).

The specific species of residues detected are listed in Table 3. There are several specific groups of residues according to the EU legislation: A (1-6), B (1-3). European Union member states report all positive samples from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products including milk (EFSA, 2021).

The Slovak Republic has performed the monitoring and the targeted sampling according to the EU legislation. The number of positive samples was:

- 2013: Chloramphenicol (1 positive sample) (EFSA, 2015),
- 2012: Benzylpenicillin (Penicillin G) (1 positive sample) (EFSA, 2014),
- 2009: Chloramphenicol (1 positive sample) (EFSA, 2011).

Positive samples present in Table 1 are not included in these reports (Table 2), because they were detected in the self-control by dairies and were not sampled as a part of the targeted monitoring performed by the Veterinary and Food Administration of the Slovak Republic.

The monitoring system in Slovakia is functional and will detect possible contamination of milk with residues of veterinary drugs. Dairies in Slovakia process only milk that does not contain residues of veterinary drugs.

Table 3 Overview of the inhibitory substances detected in milk in EU 2015 – 2019.

Year	Overview of the substances detected	Source
2019	In group A, there were three non-compliant samples and non-compliant results reported in group A6 (chloramphenicol), by three countries. For antibacterials (B1), seven countries reported a total of 11 non-compliant samples and results. In the group B2, there were 5 non-compliant samples and results for anthelmintics (B2a) and 19 non-compliant samples and results for NSAIDs (B2c), reported by three and eight countries, respectively. In the group B3, there were three non-compliant samples and results for mycotoxins (B3d), relating to Aflatoxin M1, reported by two countries. For organochlorine compounds, including PCBs (B3a) there was one non-compliant sample (2 non-compliant results) reported by one country. More information on the specific substances identified and the number of non-compliant results reported by each country is given in Appendix A. Detected substances: Chloramphenicol, Aminosidin (Paromycin, Paromomycin), Amoxicillin, Ampicillin, Benzylpenicillin (Penicillin G), Dihydrostreptomycin, Doxycycline, Rifaximin, Closantel, Ivermectin, Moxidectin, Oxytetracycline, Acetaminophen (Paracetamol), Antipyrin-4-Hydroxy, Diclofen (Diclofenac), Salicylic acid, TEQ Dioxin-like PCBs LB, Aflatoxin M1.	(EFSA, 2021)
2018	In the group A, there were two non-compliant samples (two non-compliant results) for group A6 (chloramphenicol), reported by two Member States. For antibacterials (B1), four Member States reported a total of four non-compliant samples (and results). In the group B2, there were 18 non-compliant samples and results: 6 for anthelmintics (B2a) and 12 for NSAIDs (B2c). In the group B3, there were three non-compliant samples and results for chemical elements (B3c), relating to lead, reported by two Member States. More information on the specific substances identified and the number of non-compliant results reported by each Member State is given in Appendix A. Detected substances: Chloramphenicol, Aminosidin (Paromycin, Paromomycin), Amoxicillin, Cloxacillin, Oxytetracycline, Closantel, Ivermectin, Albendazole, Sulphoxide, Albendazole sulphone, Albendazole 2-amino sulphone, Diclofen (Diclofenac), Salicylic acid, Lead (Pb).	(EFSA, 2020)
2017	In the group A, there were two non-compliant samples (five non-compliant results) in group A6 reported by one Member State (one result each for AHD, AMOZ, AOZ, chloramphenicol and SEM). For antibacterials (B1), seven Member States reported a total of 19 non-compliant samples (20 non-compliant results). In the group B2, there were 54 non-compliant samples and results: 11 for anthelmintics (B2a) and 43 for NSAIDs (B2c). In the group B3, there were 11 non-compliant samples and results for mycotoxins (B3d) (all aflatoxin M1). More information on the specific substances identified and the number of non-compliant results reported by each Member State is given in Appendix A. Detected substances: AHD (1-aminohydantoin), AMOZ (5-methylmorpholino-3-amino-2-oxazolidone), AOZ (3-amino-2-oxazolidone), Chloramphenicol, SEM (semicarbazide), Ampicillin, Benzylpenicillin (Penicillin G), Cefalonium, Ciprofloxacin, Cloxacillin, Doxycycline, Florfenico, Oxytetracycline and its 4-epimer, tetracycline and its 4-epimer, Trimethoprim, Tulathromycin, Clorsulon, Closantel, Ivermectin, Levamisole, Nitroxinil, Ketotriclabendazole, Oxfendazole sulphone, Triclabendazolsulfon, Diclofen (Diclofenac), Salicylic acid, Aflatoxin M1.	(EFSA, 2019)
2016	Detected substances: Chloramphenicol, Hydroxymetronidazol (MNZOH), Amoxicillin, Benzylpenicillin (Penicillin G), Danofloxacin, Gentamicin, Tetracycline, Tilmicosin, Ivermectin, Triclabendazole, Diclofen (Diclofenac), Salicylic acid, 2,4-Dichlorophenoxybutyric acid, Lead, Aflatoxin M1.	(EFSA, 2018)
2015	Detected substances: Chloramphenicol, Amoxicillin, Benzylpenicillin (Penicillin G), Cefalexin (Cefalexin Anhydrate), Cefquinom, Cloxacillin, Enrofloxacin, Kanamycin, Spiramycin, Tetracycline, Albendazol, Closantel, Ivermectin, Diclofen (Diclofenac), Lead Pb, Aflatoxin M1.	(EFSA, 2017)

CONCLUSION

The system of monitoring of inhibitory drug residues in raw cow's milk applied in Slovakia is effective, it can detect the presence of these substances before milk is processed in dairies. The total incidence of these substances in 2020 was 0.025% of all 12,181 samples tested. A total number of 452,731 samples have been tested over the last twenty years in central testing laboratories. The number of drug residues positive samples significantly ($p < 0.05$) decreases from 299 (2001) to 3 (2020). This is because, since about 2004, rapid tests for the detection of residues of inhibitory drug substances have become more widely used in Slovakia. The system of monitoring effectively reducing the risk resulting from the occurrence of residues of inhibitory substances in raw cow's milk on human health. As it is not possible to test

samples from all milk deliveries in central testing laboratories, the responsibility for control and testing remains on the dairies. Every supply of raw cow's milk is tested for the presence of residues of inhibitory substances in each dairy company in Slovakia. The role of the State Veterinary and Food Administration of the Slovak Republic is to monitor and control whether food business operators implement measures to prevent the placing on the market of milk if it contains residues of inhibitory substances. This institution annually compiles the National Plan for the Control of Residues in Live Animals and Products of Animal Origin following the applicable legislation. Only the three positive samples were detected in targeted monitoring during the last ten years.

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This article does not contain any studies that would require an ethical statement.

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