sciendo

DOI: 10.2478/fv-2021-0038

FOLIA VETERINARIA, 65, 4: 57—66, 2021

SELECTION OF THE MOST RELIABLE METHOD FOR THE ANALYSIS OF INHIBITORY SUBSTANCES IN RAW AND SKIMMED MILK

Hriciková, S., Kožárová, I.

Department of Food Hygiene, Technology and Safety,
University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
Slovakia

simona.hricikova@student.uvlf.sk

ABSTRACT

Milk used for human consumption must comply with the European Union legislative requirements for residues of inhibitory substances in milk, the values of which must not exceed the established maximum residue limit. In order to ensure the quality and safety of milk and milk products placed on the market, the presence of residues of inhibitory substances should be monitored and verified. The aim of our study was to select the most reliable method for the analysis of residues of inhibitory substances in milk. In the search for the most reliable method, a total of 49 milk samples were tested in the form of raw milk, skimmed milk and skimmed-milk powder throughout the agri-food chain. For comparison, the microbial inhibition tests Eclipse 50, Eclipse Farm, Explorer 2.0, Delvotest®, Premi®Test and the fast receptor screening test TwinSensor were used. The most relevant results were obtained by the Eclipse 50 and Eclipse Farm tests, the reliability of which were also confirmed by the Explorer 2.0 and Premi®Test tests. Moreover, according to the State Veterinary and Food Administration of the Slovak Republic, Eclipse 50 is an official reference method for the determination of residues of inhibitory substances in milk. Therefore, we can only state that of all the methods used, the Eclipse 50 seems to be the most reliable for routine control analysis of residues of inhibitory substances in all types of milk.

Key words: analysis; inhibitory substances; milk; analysis; screening tests

INTRODUCTION

Milk and milk products as a source of nutrients, probiotics and prebiotics, vitamins and minerals, are widely consumed worldwide. They have become a major part of a balanced diet and are important for people's nutrition and health. There are several different types of milk, e.g. raw milk, whole milk, semi-skimmed, skimmed milk, powdered milk like skimmed milk powder, etc. "Raw milk" means milk produced by the secretion of the mammary gland of farmed animals which has not been heated

to more than 40 °C or undergone any treatment that has an equivalent effect [15]. Skimmed milk is made by removing the fat components by centrifugation. To obtain skimmed milk powder, the process involves evaporating water and drying at 94 °C.

However, milk is exposed to and gets contaminated with various types of chemical contaminants. One of these undesirable contaminants in milk and milk products are residues of pharmacologically active substances. 'Residues of pharmacologically active substances' means all pharmacologically active substances, expressed in $mg.kg^{-1}$ or $\mu g.kg^{-1}$ on a fresh weight basis, whether active substances, excipients or degradation products, and their metabolites which remain in food obtained from animals [14]. Inhibitors, especially antibiotics, are currently widely used in humans as well as other animals.

The use of antibiotics in livestock is of great importance in the treatment of bacterial infections, but also to ensure the quality of life of animals, but their use has a significant impact on the quality and safety of animal products. Approximately 63,000 tons of antibiotics are used in livestock worldwide each year [18]. They are used for therapeutic and prophylactic purposes [10], but also for growth promotion [2]. The global use of antimicrobials in animals is twice as high as in humans [1].

Many studies have shown that significant amounts (30—70 %) of antibiotics are released unchanged, i. e., with potential antimicrobial activity in the environment [10]. When released into the environment, most antibiotics are persistent and biologically active [17]. The main risks we should consider when using antibiotics are residues of inhibitory substances and the development of microbial resistance [7]. The World Health Organization [19] emphasizes that bacterial resistance to antibiotics is rising to dangerously high levels in all parts of the world. Globally, new mechanisms of resistance are emerging and spreading that threaten our ability to treat common infectious diseases. Various alternatives to antibiotics (plant-derived substances and probiotics) can be beneficial in the fight against bacterial resistance [3].

The residues of various antibiotics are associated with many types of allergic reactions, especially in the case of penicillin. Antibiotic residues have potential carcinogenic effects by interacting with DNA and RNA [5]; and a mutagenic effect that can cause mutation of the DNA molecule or damage to chromosomes [8]. Such mutations can manifest itself as infertility for an example [16]. In the newborn, various congenital anomalies may occur due to long-term exposure to antibiotic residues during pregnancy [5]. The use of broad-spectrum antibiotics can destroy a variety of bacteria in the gut, including non-pathogenic organisms, which can disrupt the normal intestinal microflora [16]. Last but not least, the existence of antibiotic residues in milk, even in very low concentrations, is very worrying in the dairy industry. Antibiotic residues can interfere with the fermentation process during cheese and yogurt production by inhibiting starter cultures [5].

Milk used for human consumption must comply with the European Union requirements for residues of inhibitory substances in milk, the values of which must not exceed a maximum residue limit (MRL). MRL is the maximum concentration of a residue of a pharmacologically active substance which may be permitted in food of animal origin [14]. On 15 March 2019, Commission Implementing Regulation (EU) No. 2019/627 laid down uniform practical arrangements for carrying out official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and measures for the monitoring of certain substances and their residues in live animals and animal products in accordance with Council Directive 96/23/EC of 29 April 1996. Council Directive 96/23/EC provides, inter alia, that antibacterial substances, including sulphonamides and quinolones, are to be monitored in milk. For this purpose, several methods are currently available, such as e.g., chromatographic, immunological, microbiological and others.

An effective screening method must be inexpensive and capable of high-throughput, and able to effectively identify potentially non-compliant samples from a large number of negative samples. Microbial inhibition tests were the first methods used to detect antibiotic residues and are still used. They are cost-effective and, in contrast to e.g., immunological or receptor-based assays, they have the potential to cover the entire antibiotic spectrum within one test. This format is commonly used in routine milk screening [13].

Microbial inhibition tests are available that can detect a wide range of antibacterial substances in milk, such as betalactams, tetracyclines, sulfonamides, macrolides, aminoglycosides and trimethoprim. These tests are available in tube (or vial, or ampoule) format and are easy to use, affordable and the test result is readable within two to three hours. The tubes contain an agar medium inoculated

with (spores of) a sensitive test bacterium, supplemented with a pH or redox indicator. At the appropriate temperature, the bacteria start to grow and produce acid, which will cause a color change. The presence of antimicrobial residues will prevent or delay bacterial growth, and thus is indicated by the absence or delay of the color change. This group of tests includes, e. g. Delvotest or Eclipse test. Immune-receptor tests are competitive tests involving specific receptors with high affinity to specific antibiotic groups. They usually comprise of a lateral flow strips which are read by eye or an incubator Reader. They are very fast and the test result is readable within 5 to 10 minutes. Therefore, they are suitable for initial rapid antibiotic screening. However, these assays are generally limited to the detection of certain antibiotics, such as beta-lactams or tetracyclines. From this type of tests are available, e.g. TwinSensor, BetaStar, AuroFlow, or BT Scan [11, 13].

In order to ensure and verify the safety of milk and milk products without the presence of antibiotic residues, the aim of our study was to select the most reliable method for the routine analysis of inhibitory substances in various types of milk.

MATERIALS AND METHODS

As part of the search for the most reliable method, a total of 49 cow's milk samples in the form of raw milk, skimmed milk and skimmed milk powder. The samples were provided to us by milk processing plant or were purchased from retail chains. The tested raw cow's milk was obtained from five different dairy farms from Eastern Slovakia. Samples were examined using microbial inhibition tests: Eclipse 50 (Zeulab, Zaragoza, Spain), Eclipse Farm (Zeulab, Zaragoza, Spain), Explorer 2.0 (Zeulab, Zaragoza, Spain), Delvotest® (DSM, Food Specialties, Delft, The Netherlands) and Premi®Test (R-Biopharm AG, Darmstadt, Germany), as well as TwinSensor (Unisensor s.a., Ougrée, Belgium) rapid receptor screening assay.

Preparation of samples

Raw and skimmed milk was stored in a freezer at –18 °C and immediately before testing heated to a temperature of 40 °C for 20 minutes. The skimmed milk powder was treated with demineralized water in a ratio of 1 : 5 before the analysis.

Procedure

- **Eclipse 50, Eclipse Farm, Explorer 2.0, Delvotest®, Premi®Test:** Milk samples were transferred to individual ampoules or microtiter plates as follows: 50 µl (Eclipse 50) or 100 µl (Eclipse Farm, Explorer 2.0, Delvotest®, Premi®Test). A negative control using a milk sample that does not contain any inhibitory substances was also used to check the test accuracy. The wells were thoroughly sealed with an adhesive foil provided by the tests and placed in an incubator (Labnet Accublock Digital Dry Bath D 1200, Labnet, Edison, USA). The samples were incubated at 65 °C ± 2 °C for 2 to 3 hours depending on the test. The colour change of the negative control was monitored. When the colour of the negative control changed to yellow, the incubation was terminated. All samples were tested twice to verify the test result.
- **TwinSensor:** The test protocol as set by the kit manufacturer was followed. Milk samples (200 µl) were applied to microwells containing the lyophilized reagent and incubated in a HeatSensor (Unisensor, SA, Ougrée, Belgium) for 3 minutes at 40 °C. After the first three minutes, the strips were placed in the incubator above the corresponding milk samples and incubate for another 3 minutes at 40 °C. At the end of the incubation, the test strips were prepared to evaluate the results.

Evaluation of results

Eclipse 50, Eclipse Farm, Explorer 2.0, Delvotest®, Premi®Test

The interpretation of the results is possible visually or using an e-Reader. The yellow colour of the solid medium indicates that no antibiotics are present above the detection limit of the test and the milk is suitable for further processing. The purple colour of the solid medium indicates the presence of antibiotics whose concentration exceeds the detection limit of the test. This milk must not be used for further processing. Yellow/purple coloration of the solid medium indicates the presence of antibiotics at the level of the detection limit of the test (Figure 1).

Instrumental reading with e-Reader

Explorer 2.0 and Eclipse Farm tests are also compatible with the equipment e-Reader (Zeulab, Zaragoza, Spain). The e-Reader eliminates any doubts and provides numerical and qualitative evaluation of the samples, thus auto-

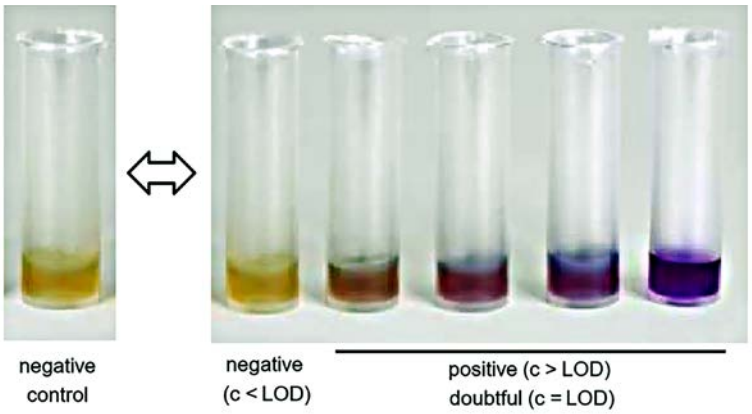


Fig. 1. Evaluation of microbial inhibition tests
https://www.bioscience.com.sg/testing-for-antibiotics-in-food-and-feed-industries/

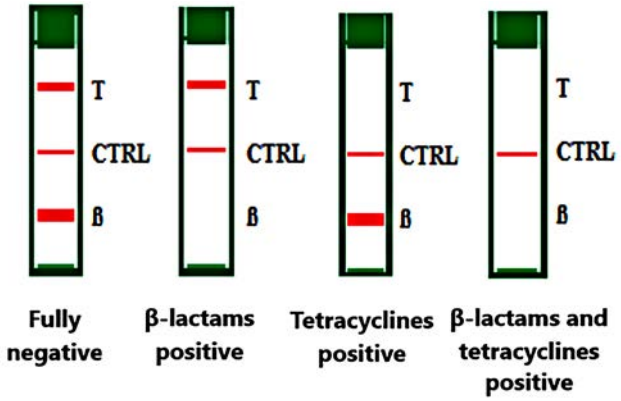


Fig. 2. Evaluation of TwinSensor

mating the process and standardised results. In addition, the instrument detects the colour change of the negative control by spectrophotometric measurement, determines the cut-off value and automatically terminates the incubation of the samples. The main advantage of combining tests with the e-Reader is the objectivity of the results, which ultimately saves time and reduces the number of erroneous (false positive/false negative) results.

TwinSensor

Test results can be evaluated visually or with the ReadSensor 2 device (Unisensor SA, Ougrée, Belgium). Visual interpretation of the result is obtained by comparing the red colour intensity of each test line with the control (CTRL) line. If the test line is darker than the CTRL line, the sample is considered negative for the antibiotic concerned, or it contains a concentration of antibiotic below the detection limit of the test. If the test line is of the same intensity, or if it is a lighter than the CTRL line, the sample is considered positive for the antibiotic concerned, and it contains an antibiotic concentration equal to, or above the detection limit of the test (Figure 2). ReadSensor quantifies, interprets, and records test strip results.

RESULTS

In the first round of testing, a total of 20 samples of raw cow's milk and a negative control sample were analysed using a broad spectrum microbial inhibition tests Eclipse 50, Eclipse Farm, Explorer 2.0, Delvotest®, Premi®Test and a narrow-spectrum test TwinSensor. Sample was tested in duplicate. As we can see from Table 1, Delvotest® (Fig. 3) and TwinSensor (Fig. 4) tests showed positive re-

Table 1. Results of testing 20 raw milk samples using 6 different tests

Sample ↓	Test name					
	Eclipse 50	Eclipse Farm	Delvotest®	Explorer 2.0	Premi®Test	TwinSensor
1	–	–	+	–	–	+
2	–	–	+	–	–	+
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	–	–	–	–	–	–
7	–	–	–	–	–	–
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	–	–	–	–	–	–
11	–	–	–	–	–	–
12	–	–	–	–	–	–
13	–	–	–	–	–	–
14	–	–	–	–	–	–
15	–	–	–	–	–	–
16	–	–	–	–	–	–
17	–	–	–	–	–	–
18	–	–	–	–	–	–
19	–	–	–	–	–	–
20	–	–	–	–	–	–
Negative control	–	–	–	–	–	–

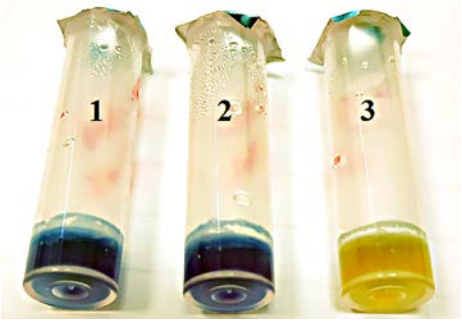


Fig. 3. Delvotest® result

sults in sample No. 1 and 2. The Eclipse 50, Eclipse Farm, Premi®Test and Explorer 2.0 tests showed negative results for all 20 examined milk samples.

As shown in Figure 4, the results of TwinSensor test indicated the presence of β-lactam antibiotics in both samples (No. 1 and 2). The test failed to confirm the presence of tetracyclines. The results of the visual evaluation were also confirmed by the ReadSensor 2 device (Figure 5).

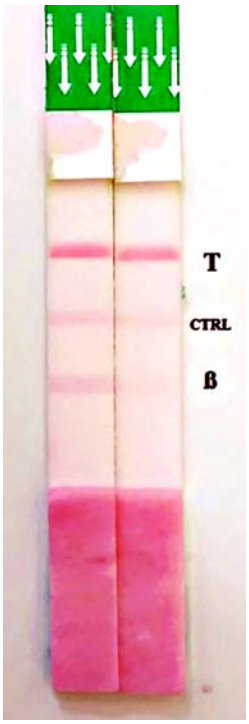


Fig. 4. TwinSensor test result

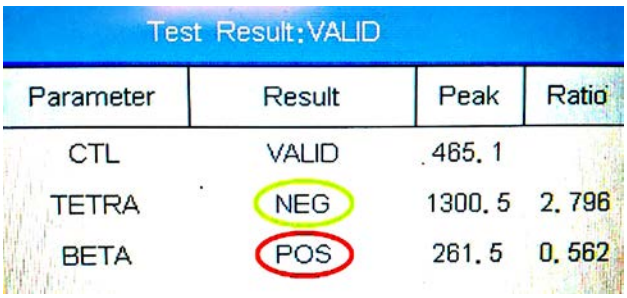


Fig. 5. TwinSensor result using the ReadSensor 2

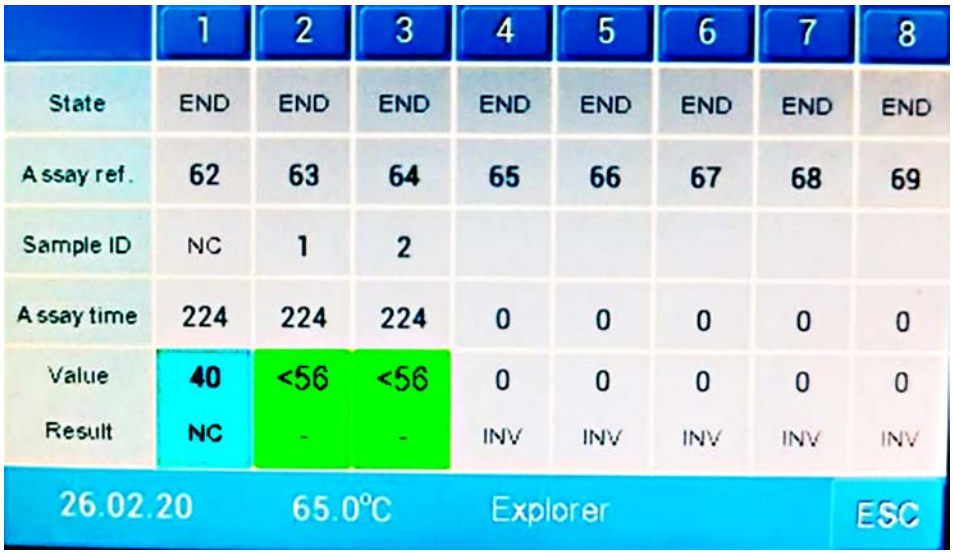


Fig. 6. Result of the Explorer 2.0 test using the e-Reader where No. 1 represents a negative control

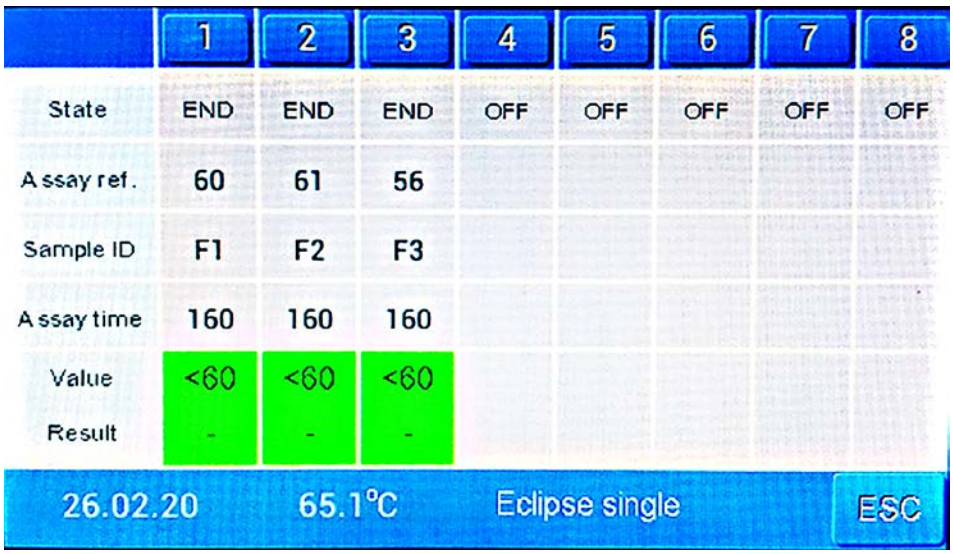


Fig. 7. Result of the EclipseFarm test using the e-Reader where No. 1 represents a negative control

Table 2. Eclipse 50 test results

Type of milk ↓	Farm 1		Farm 2		Farm 3	
	No.		No.		No.	
Raw milk	1	–	4	–	7	–
Skimmed milk	2	–	5	–	8	–
Skimmed milk powder	3	–	6	–	9	–
Negative control						
Demineralised water		–	**Rajo low-fat milk 0.5 %**		–	

In addition to the visual evaluation in the case of samples No. 1 and 2 (positive results), we subsequently used the e-Reader for the instrumental reading to confirm or refute the positive results. The following analysis was done by the Explorer 2.0 (Figure 6) and Eclipse Farm (Figure 7) test compatible with e-Reader. The results proved negativity in both samples results.

In the second round of testing, another 9 milk samples in the type of raw cow's milk, skimmed milk and skimmed milk powder provided to us by milk processing plant as control samples taken during routine milk processing operations, were analysed. From all tests used in the first round of testing and their results obtained, the Eclipse 50 microbial inhibition test was only used. Demineralized water and Rajo low-fat milk with a fat content of 0.5 % were used as a negative control. All samples showed a negative result, as we can see from Table 2.

For verification of the presence of residues of inhibitory substances in skimmed milk offered to consumers in the retail network, a total of 20 samples of skimmed milk with a fat content of 0.5 % were purchased from various retail chains and analysed by the Eclipse 50 test. In addition, according to the State Veterinary and Food Administration of the Slovak Republic, the Eclipse 50 test is the official reference method for detection of the presence of inhibitory substances in milk. When detecting residues of inhibitory substances in skimmed milk with a fat content of 0.5 %, the Eclipse 50 test confirmed our expected results. None of the 20 examined skimmed milk samples contained residues of inhibitory substances above the permitted MRL. We are very pleased with this test result, which indicates the required safety of milk that is sold for human consumption.

DISCUSSION

The European Food Safety Authority report [9] summarises the data collected in 2019 on the presence of residues of veterinary medicinal products and certain substances in live animals and animal products in the European Union, Iceland and Norway. A total of 671,642 samples were reported to the European Commission by the 27 out of the 28 EU Member States, Iceland and Norway. They consisted of 368,594 targeted samples and 5,016 suspect samples reported under Council Directive 96/23/EC, and of 2,342 samples collected at import and 295,690 samples collected in the framework of programs developed under the national legislation. The majority of countries fulfilled the minimum requirements for sampling frequency laid down in Council Directive 96/23/EC and in Commission Decision 97/747/EC. Overall, the percentage of non-compliant samples in 2019 (0.32 %) was comparable to the previous 11 years (0.25 %—0.37 %). Slight decreases were noted in 2019, for resorcylic acid lactones, prohibited substances, antibacterials, anticoccidials, and dyes, compared to 2017 and 2018. For Group B1 (antibacterials), 0.14 % of the samples analysed under the Directive 96/23/EC monitoring were non-compliant.

B a c h i et al. [16] in their survey "Antibiotic residues in milk: past, present and future" found that residues of inhibitory substances in milk are often and largely detected worldwide. The world's highest number of publications on the detection of residues of inhibitory substances in milk come from Europe: 105 (46.88 %), followed by Asia: 77 (34.38 %), South America: 18 (8.04 %), North America: 16 (7.14 %) and Africa: 8 (3.57 %). Within Europe, Spain

leads with 30 (13.39 %) publications and Germany with 11 (4.91 %) published works. Cow's milk is most often tested, in a total of 193 publications (86.16 %), followed by sheep's milk: 9 (8.48 %) and goat's milk: 14 (6.25 %). Residues of β-lactam antibiotics were detected to the highest extent, in 133 (36.54 %) cases, tetracyclines: 51 (14.01 %), fluoroquinolones: 49 (13.46 %), sulfonamides: 46 (12.64 %) and aminoglycosides: 38 (10.44 %). The data suggest that the use of β-lactam antibiotics in dairy cows is increasing every day. In 16.96 % of detection cases, microbiological methods were used to determine residues of inhibitory substances in milk. Over the last 10 years, the number of publications has increased by almost 30 %, which indicates a growing trend in the detection of antibiotics in milk.

There are several studies on the use of microbial inhibition tests for the screening of antibiotic residues in cow, sheep and goat milk. B e l t r á n et al. [4] tested the performance of the current microbial tests, such as the BRT MRL (Analytik in Milch Produktions-und Vertriebs-GmbH, Munich, Germany), Delvotest MCS SP-NT (DSM Food Specialties, Delft, the Netherlands), Delvotest MCS Accelerator (DSM Food Specialties), and Eclipse 100 (Zeulab, Zaragoza, Spain) for the screening of antibiotics in milk. They confirmed that the microbial inhibition tests are efficient to detect β-lactams and other non-beta-lactam drugs such as neomycin, tylosin, sulfadiazine and sulfadimethoxine in raw milk from small ruminants. However, in spite of the improvements made in these tests in the last decade, they continue to be inefficient for the detection of other drugs, such as quinolones and tetracyclines, at safety levels. Therefore, the periodic use of more sensitive tests towards these substances would be convenient to widen the detection range in screening and guarantee the quality of milk and dairy products from small ruminants.

B i o n et al. [6] evaluated Delvotest® T for its ability to detect 27 antibiotic residues in raw cow's milk, skimmed milk and whole milk powder. The test showed a false negative result for nafcillin, oxytetracycline, tetracycline, rifaximin and sulfadiazine compounds.

In the research of M a t a, S a n z, R a z q u i n [12], Eclipse Farm test was verified in combination with an e-Reader. Detection limits for penicillins, cephalosporins, tetracyclines, sulfonamides, macrolides and aminoglycosides were evaluated. All residues were detected at or below the MRL. It was also shown that the course of the method was not affected by changes in the procedure (sample volume, incubation temperature, test dose). Usability has been demonstrated in raw cow's milk regardless of fat and protein content or high somatic cell content. Eclipse Farm in combination with e-Reader has proven to be a valuable tool for screening a wide range of antimicrobial residues in raw milk. The test is sensitive and easy to use. e-Reader simplifies analysis and increases the accuracy of results, interprets results in an objective way.

In our study we used Eclipse 50, Eclipse Farm coupled to e-Reader, Explorer 2.0, Delvotest® and Premi®Test as the microbial inhibition tests, and TwinSensor coupled to ReadSensor 2 as the immune-receptor test for analysis of residues of inhibitory substances in a total of 49 samples of raw milk, skimmed milk and skimmed milk powder. We considered that the most relevant and uniform results were obtained by the Eclipse 50 and Eclipse Farm tests, the reliability of which was also confirmed by the Explorer 2.0 and Premi®Test.

All these microbiological tests combine the principle of agar diffusion tests with colour change of a pH indicator from blue/violet to yellow due to the active metabolism of the test strain Bacillus stearothremophillus var. calidolactis at the absence of inhibitory substance in the sample. Bacillus stearothermophilus var. calidolactis is generally considered the most susceptible microbial strain for broad-spectrum analysis of antibiotic residues in various food matrices of animal origin such as milk, but also meat, eggs, fish, shrimps and honey.

All these tests are able to detect a broad-spectrum of antibiotics such as b-lactam, aminoglycosides, tetracyclines, sulphonamides, macrolides, lincosamides and quinolones at the level of MRL. This ability clearly favours them over tests that have a narrow spectrum of activity, but the importance of the competitive immune-receptor tests with a narrow spectrum of activity cannot be questioned, whereas it is precisely these tests which are the tests of first choice for verifying the presence of residues of a specific group/groups of antibiotics in milk.

CONCLUSIONS

The aim of our study was to select the most reliable method for the analysis of residues of inhibitory substances in various types of milk. In terms of cost, efficacy, spectrum of detectable substances and time consuming, rapid receptor screening tests or microbial inhibition tests are

mostly used on the dairy farm and in the milk processing plant. From all tests used, only two tests, the Delvotest® and the TwinSensor detected positive results in the same two raw milk samples with the positivity for beta-lactam antibiotics. Further testing of both positive samples with the Eclipse 50 and the Eclipse Farm coupled to instrumental reading with an e-Reader do not confirmed the positive result obtained. Positive results probably occurred due to the higher sensitivity of both methods used compared to other tests. Such over-sensitivity of screening tests is not desirable for residue control purposes, as such screening tests should detect the presence of a substance or class of substances at the level of interest (compliance with EU MRL) without the risk of false-positive results. The Eclipse 50 and Eclipse Farm tests coupled to e-Reader proved to be the more relevant tool in residue analysis. e-Reader provides an objective and accurate result in an automatic way, but the analysis is instrumentally demanding and expensive. Due to the technical and economical aspect of residue testing in milk, the use of Eclipse 50 is more practical and cost-effective for the routine control of residues of inhibitory substances in all types of milk.

ACKNOWLEDGEMENT

Our thanks belong to Syráreň Bel Slovensko, a. s., for providing milk samples and supporting the implementation of experimental work.

REFERENCES

1. **Aarestrup, F., 2012:** Sustainable farming: Get pigs off antibiotics. *Nature*. 486, 465—6. DOI: 10.1038/486465a.
2. **Arikan, O. A., 2008:** Degradation and metabolization of chlortetracycline during the anaerobic digestion of manure from medicated calves. *J. Hazard. Mater.*, 158, 485—90. DOI: 10.1016/j.jhazmat.2008.01.096.
3. **Bacanlı, M., Başaran, N., 2019:** Importance of antibiotic residues in animal food. *Food Chem. Toxicol.*, 125, 462—6. DOI: 10.1016/j.fct.2019.01.033.
4. **Beltrán, M. C., Berruga, M. I., Molina, A., Althaus, R. L., Molina, M. P., 2016:** Performance of current microbial tests for screening antibiotics in sheep and goat milk. *Int. Dairy J.*, 41, 13—15. DOI: 10.1016/j.idairyj.2014.09.007.
5. **Beyene, T., 2016:** Veterinary drug residues in food-animal products: Its risk factors and potential effects on public health. *J. Vet. Sci. Technol.*, 7, 1. DOI: 10.4172/2157-7579.1000285.
6. **Bion, C., Beck Henzelin, A., Qu, Y., Pizzocri, G., Bolzoni, G., Buffoli, E., 2016:** Analysis of 27 antibiotic residues in raw cow's milk and milk-based products—validation of Delvotest® T. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.*, 33, 54—59. DOI: 10.1080/19440049.2015.1104731.
7. **Carlet, J., Jarlier, V., Harbarth, S., Voss, A., Goossens, H., Pittet, D., et al., 2012:** Ready for a world without antibiotics? The Pensières Antibiotic Resistance Call to Action. *Antimicrob. Resist. Infect. Control*, 1, 11. DOI: 10.1186/2047-2994-1-11.
8. **Conzuelo, F., Ruiz-Valdepeñas Montiel, V., Campuzano, S., Gamella, M., Torrente-Rodríguez, R. M., Reviejo, A. J., et al., 2014:** Rapid screening of multiple antibiotic residues in milk using disposable amperometric magnetosensors. *Anal. Chim. Acta*, 820, 32—38. DOI: 10.1016/j.aca.2014.03.005.
9. **European Food Safety Authority:** *Report for 2019 on the Results From the Monitoring of Veterinary Medicinal Product Residues and Other Substances in Live Animals and Animal Products*. https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2021.EN-1997. Updated March, 2021. Accessed October 18, 2021.
10. **Kümmerer, K., 2010:** Pharmaceuticals in the environment. *Annu. Rev. Environ. Resour.*, 35, 57—75. DOI: 10.1146/annurev-environ-052809-161223.
11. **Labtek Services:** *Antibiotics in Milk Testing Review*. https://labtekservices.co.uk/antibiotic-residue-testing-in-milk/antibiotic-milk-testing-review/. Updated July 2, 2021. Accessed October 2, 2021.
12. **Mata, L., Sanz, D., Razquin, P., 2016:** Performance of Eclipse Farm Test coupled to e-Reader for antibiotic residues detection in raw milk. *Food Anal. Methods*, 9, 407—410. DOI: 10.1007/s12161-015-0210-y.
13. **Pikkemaat, M. G., 2009:** Microbial screening methods for detection of antibiotic residues in slaughter animals. *Anal. Bioanal. Chem.*, 395, 893—905. DOI: 10.1007/s00216-009-2841-6.
14. **Regulation (EC) No. 470/2009:** *of the European Parliament and of the Council of 6 May 2009 Laying Down Community Procedures for the Establishment of Residue Limits of Pharmacologically Active Substances in Foodstuffs of Animal Origin, repealing Council Regulation (EEC) No. 2377/90 and amending Directive 2001/82/EC of the European Parliament and of*

the Council and Regulation (EC) No. 726/2004 of the European Parliament and of the Council (Text with EEA relevance) http://data.europa.eu/eli/reg/2009/470/oj/eng. Updated June 16, 2009. Accessed October 15, 2021.

15. **Regulation (EC) No. 853/2004:** *of the European Parliament and of the Council of 29 April 2004 Laying Down Specific Hygiene Rules for Food of Animal Origin*. http://data.europa.eu/eli/reg/2004/853/oj/eng. Updated April 30, 2004. Accessed October 15, 2021.
16. **Sachi, S., Ferdous, J., Sikder, M. H., Azizul Karim Hussani, S. M., 2019:** Antibiotic residues in milk: Past, present, and future. *J. Adv. Vet. Anim. Res.*, 6, 315—332. DOI: 10.5455/javar.2019.f350.
17. **Sarmah, A. K., Meyer, M. T., Boxall, A. B. A., 2006:** A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*, 65, 725—59. DOI: 10.1016/j.chemosphere.2006.03.026.
18. **Van Boeckel, T. P., Brower, C., Gilbert M., Grenfell, B. T., Levin, S. A., Robinson, T. P., et al., 2015:** Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA*, 112, 5649—54. DOI: 10.1073/pnas.1503141112.
19. **World Health Organization:** *Antibiotic Resistance*. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Updated July 31, 2020. Accessed October 5, 2021.

Received August 27, 2021
Accepted October 29, 2021