

## DETECTION OF ANTIBIOTICS IN THE MILK DEPENDING ON THE WAY OF DRUG APPLICATION

Boboš S.F., Nešić M.J., Radinović M.Ž.

Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad,  
Serbia

*In veterinary practice, antibiotics are used in local or parenteral treatment of animals. Excretion of antibiotics is usually done by milk and urine. Antibiotics residue in the milk of animals is possible to prove a few hours after application, during the withdrawal period, sometimes longer. In our tests, we followed the presence of penicillin residue in milk using Charm MRL™ Test and SNAP Test after intramuscular and intramammary application. In cows with intramammary application, we were testing the excretion of residue from treated quarter and from the other three udder quarters. Measurements were made on 24 hours during 5 days. The results of our investigation by local application indicate that the concentration of antibiotics residue in milk is the highest in treated quarter and much lower in the other 3 quarters. Intramuscular application of penicillin result the high values of concentration up to 48 hours per application, and then comes to the fall of concentration. Comparing the results of two tests we observe that SNAP Test is more sensitive, it gives positive results after 72 hours, when the Charm MRL™ Test gives negative results. The results of investigations indicate that it is necessary to exert control on the antibiotics residue in the milk after withdrawal period, no matter which test is applied.*

**Introduction.** In veterinary practice, antibiotics are used in local or parenteral treatment of animals. Excretion of antibiotics is usually done by milk and urine. Antibiotics residue in the milk of animals is possible to prove a few hours after application, during the withdrawal period, sometimes longer.

**Material and methods.** In our tests, we followed the presence of penicillin residue in milk using Charm MRL™ Test and SNAP Test after intramuscular and intramammary application.

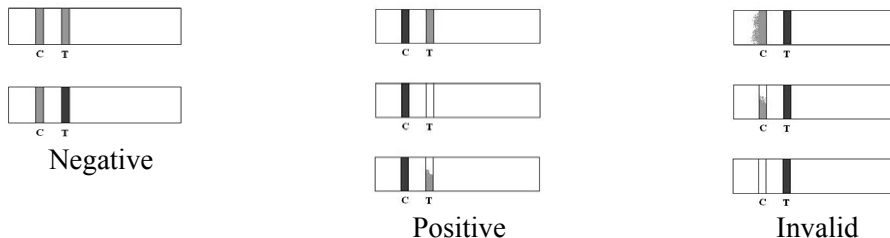
Most frequently used natural penicillin is penicillin G (benzyl-penicillin). It is the first penicillin that was introduced in the therapy. After the introduction, it showed to great results in the fight against Gram-positive bacteria, especially against Staphylococci, Streptococci, and Gram-negative Neiseria.

Withdrawal period after intramammary application is 72 hours, and after intramuscular it is also 72 hours.

First group of cows is treated with intramuscularly applied 4,000,000 I.U. of penicillin G, what represents the average therapeutic dose. Second group of cows is treated with 300,000 I.U. penicillin G locally, what also represents the average therapeutic dose. The drug is applied intramammary in the right front quarter.

In cows with intramammary application, we were testing the excretion of residue from treated quarter and from the other three udder quarters. Measurements were made on 24 hours during 5 days, using Charm MRL™ Test and SNAP Test.

Charm MRL™ Test is a quick one step receptor analysis. Charm MRL™ Test strip is read after 8 minutes incubation on 56°C in ROSA incubator.



**Scheme 1.** Interpretation of Charm Test results (negative, positive, invalid)

SNAP antibiotic residue test has two steps. SNAP results are easy to interpret – by comparing the sample spots to the control spot. With SNAPshot Reader one can get quantitative results.

**Results and discussion.** The results of our investigation by local application indicate that the concentration of antibiotic residues in milk is the highest in treated quarter and much lower in the other 3 quarters.

**Table 1** – SNAP and Charm Test results after intramammary application

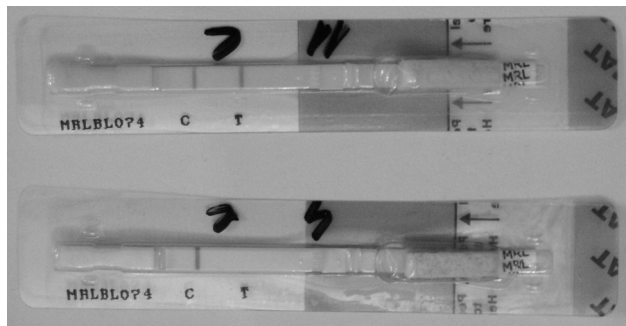
Milk from	24 h		48 h		72 h		96 h		120 h	
	Snap	Charm	Snap	Charm	Snap	Charm	Snap	Charm	Snap	Charm
treated quarter	>10	+	>10	+	>10	+	2.32	+	1.40	~
other 3 quarters	2.30	+	1.19	+	1.24	-	1.21	-	1.69	-

Intramuscular application of penicillin result the high values of concentration up to 48 hours per application, and then comes to the fall of concentration.

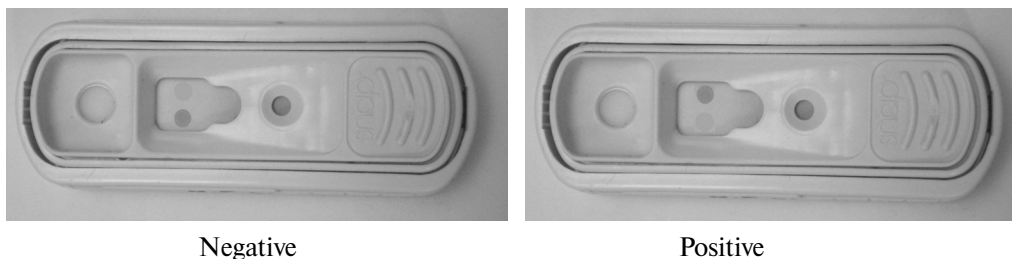
**Table 2** – SNAP and Charm Test results after intramuscular application

Milk from	24 h		48 h		72 h		96 h		120 h	
	Snap	Charm	Snap	Charm	Snap	Charm	Snap	Charm	Snap	Charm
all 4 quaters	7.08	+	>10	+	3.03	+	2.09	-	1.38	-

SNAP Test gives positive results after 72 hours, when the Charm MRL™ Test gives negative results.



**Picture 1.** Negative (up) and positive (down) Charm MRL™ Test strip



Negative

Positive

**Picture 2.** Negative (left) and positive (right) SNAP Test results

**Conclusions.** Comparing the results of two tests we observe that SNAP Test is more sensitive, it gives positive results after 72 hours, when the Charm MRL™ Test gives negative

results. The results of investigations indicate that it is necessary to exert control on the antibiotics residue in the milk after withdrawal period.

#### References

1. Petrović, J. Qualitative determination of antibiotic residues in milk with different screening methods and bio test [Text] / Petrović J., Čobanović K., Kovačević M., Ratajac R. // Arhiv veterinarske medicine. — 2008. — Vol. 1, № 2. — P. 63–71. 2. Pavlović, N. Koncentracija rezidua antibiotika posle mešanja kontaminiranih količina mleka [Text] = Antibiotics residues concentration after mixing contaminated volumes of milk / Pavlović N., Vuković V. // Prehrambena industrija — mleko i mlečni proizvodi. — 2008. — Vol. 19, № 1–2. — S. 105–107. 3. Vuković, V. Izračunavanje minimalne količine rezidua antibiotika u mleku [Text] = Determination of minimal concentration of antibiotic residues in milk / Vuković V., Kasalica A. // Prehrambena industrija — mleko i mlečni proizvodi. — 2008. — Vol. 19, № 1–2. — S. 108–109. 4. Short Communication: Antibiotic residues in milk following the use of intravaginal sponges for estrus synchronization in dairy ewes [Text] / Berruga M.I. [et al.] // J. Dairy Sci. — 2008. — Vol. 91. — P. 3917–3921. 5. Kang, J.H. False-positive outcome and drug residue in milk samples over withdrawal times [Text] / Kang J. H., Jin J. H., Kondo F. // J. Dairy Sci. — 2005. — Vol. 88. — P. 908–913. 6. Seymour E.H. Persistence of residues in milk following antibiotic treatment of dairy cattle [Text] / Seymour E.H., Jones G.M., McGilliard M.L. // J. Dairy Sci. — 1988. — Vol. 71. — P. 2292–2296.

### ВИЯВЛЕННЯ АНТИБИОТИКІВ У МОЛОЦІ В ЗАЛЕЖНОСТІ ВІД СПОСОБУ ЗАСТОСУВАННЯ ПРЕПАРАТУ

**Бобош С., Несіч М., Радінович М. Університет Нови Сад, Сербія**

*У статті наведені результати дослідження наявності залишку пеніциліну в молоці. Досліди проводили на коровах протягом 5 діб, використовуючи Charm MRL™ Test і SNAP Test після внутрішньом'язового та інтрамаммарного введення. У корів з інтрамаммарним застосуванням препарату виявлено виділення залишку з очищеної чверті та з інших трьох частин вимені. У результаті місцевого застосування виявлено, що концентрація залишку антибіотиків є вищою в молоці з очищеної чверті та значно нижчою в молоці з інших трьох чвертей. Внутрішньом'язове застосування пеніциліну протягом 48 годин призводить до його високої концентрації, яка згодом знижується. Порівнюючи результати двох тестів, було встановлено, що SNAP Test є більш чутливим і дає позитивні результати через 72 години, у той час як Charm MRL™ Test дає негативні результати.*

UDC 619:616.98:578.828.11:636.2

### INFECTION WITH BOVINE LEUKEMIA VIRUS: CURRENT DIAGNOSTIC METHODS AND MOLECULAR EPDEMIOLOGY

Kuźmak J., Wijaszka T.

National Veterinary Research Institute, Poland

*Bovine leukemia virus, a bovine retrovirus of worldwide distribution, is the etiological agent of enzootic bovine leucosis (EBL). The economic losses associated with EBL include the culling of infected cattle, reduced milk production, and trade restriction. In most of cattle-raising countries EBL is a notifiable disease, and official control measures include screening or monitoring and stamping out of infected animals. In this paper, we highlight the use of throughput screening assays (AGID and ELISA) and alternative methods (PCR) as well as give a new data related to the molecular epidemiology and genetic variability of BLV.*

**Introduction.** Bovine leukemia virus (BLV), an exogenous retrovirus, is the causative agent of bovine lymphosarcoma, enzootic bovine leucosis. Pathogenic potential of BLV is clear; the virus infects preferentially and transforms B lymphocytes, but has also been found in T cells, monocytes and granulocytes. Infection by BLV may remain silent clinically as an aleukemic form but about one-third of infected cattle develops persistent lymphocytosis and 5 to 10% lymphoid tumours. Nowadays lymphosarcoma cases are rarely found in most European countries, however, BLV infections are currently found in some ones. Because of different status of level of eradication of BLV in EU Member States and neighbouring countries, control of BLV is important for the eradication of infection and for the national and international animal trade. There are two main motives for application of EBL eradication programme: to decrease the economical losses and improve food-producing animals'