VALIDATION OF THE METHOD FOR MEASURING ANTIBIOTIC SUBSTANCES IN RAW MILK USING THE EVIDENCE INVESTIGATION ANALYZER

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Veterinary drugs for cattle are widely used in dairy farming, helping to fight bacterial infections. If the farm disrupts the processing schedule for animals and does not meet the time intervals between the addition of antibiotics (and other drugs) to the feed, then these drugs can get into the milk. For consumer confidence in dairy products to be consistently high, a comprehensive analysis of milk and dairy products for the presence of residual antibiotic substances in them is necessary. For these purposes, analyzers of the Evidence series for enzyme immunoassay are used, using the technology of matrix biochips and combining the latest technological advances in the field of detecting the residual content of drugs using the principles of enzyme immunoassay.

The versatile analyzer on biochips Randox Evidence Investigator is a unique device and is widely used all over the world to control incoming raw materials and finished products in the food industry. Biochip analysis technology allows you to simultaneously obtain quantitative data on the concentration of various substances using just one sample. Moreover, the results obtained are not inferior in accuracy to such methods as LC-MS/MS or ELISA, significantly surpassing them in efficiency.

In order to confirm that the method for the detection of veterinary drugs (pharmacologically active substances) in milk and dairy products using enzyme immunoassay with chemiluminescence detection using biochip technology meets the requirements for a specific intended use, it is necessary to conduct validation studies, which was the objective of our work.

The Evidence Investigator Randox analyzer was chosen as the object of the study. A brief description of the device is shown in Table 1.

Table 1 - Technical characteristics of the Evidence Investigator analyzer

Specifications	
Name	Value
Operating conditions	Temperature 16-25°C, relative humidity <80%, altitude <2000 m
Data backup methods	Burn to DVD, CD, USB stick or hard disk
Measuring principle	Chemiluminescent
External equipment	Printer, barcode scanner, media tray, thermal shaker and thermal cycler
Incubation period	Specificity of the array, 30-90 minutes
Capacity	9 biochips when loaded into the analyzer and 54 biochips when incubated in a thermal shaker
Bandwidth	up to 2376 tests per hour

The analysis scheme included the following stages:
–adding a diluent and samples;
– incubation of biochips;
–introduction of the conjugate;
–re-incubation of biochips;

–washing biochips;

–introduction of a working signal reagent$

– visualization of biochips.

During the experiment, 10 analyzes of samples of samples with a concentration of representative analytes at five levels were carried out, including a sample that did not contain representative analytes.

The method was validated according to such analytical characteristics as detection limit, repeatability, and specificity. The experiment to assess the detection limit was performed for thirteen representative analytes (danofloxacin, penicillin G, cephalexin, tetracycline, chloramphenicol, neomycin, streptomycin, erythrocin, tylosin, lincomycin, trimethoprim, sulfamoxol, dapsone) belonging to veterinarians of each target group. During the experiment, 6 analyzes of sample samples with concentrations of representative analytes were carried out. Evaluation of the limit of detection was carried out using the relationship: the proportion of positive results - mass fraction of the analyte. For all representative analytes, the difference between the value of the estimate of the detection limit obtained in the experiment and the specified test systems did not exceed the estimate of the expanded uncertainty of the mass fraction of representative analytes in the samples for the experiment.

When determining the content of antibiotics in raw milk, six samples were prepared, tests were carried out and the relative standard deviation (RSD) was calculated. The acceptance criterion is the relative standard deviation of the mean (RSD, %), which should not exceed 1.0%. The results of the experiment are shown in table 2, from which it can be seen that the RSD does not exceed 1.0%.

Table 2 –

Results of statistical processing of experimental data

Observation number.	Analyte content	$X_{av.}$	$S(x)$	RSD_x, %
1	0,21	0,16	0,064	0,41
2	0,06			
3	0,20			
4	0,17			
5	0,09			
6	0,20			

During the experiment, 2 analyzes of samples of samples containing representative non-target veterinary drugs (albendazole, dinitrocarbanilide, toltrazuril, mentonidazole) were carried out with their mass fraction 10 times higher than the permissible level of content, according to the current legislation. The research results showed that the proportion of false negative results (FPR) is equal to 0.

Thus, as a result of validation of the method for the detection of veterinary drugs (pharmacologically active substances) in milk and dairy products using enzyme-linked immunosorbent assay with chemiluminescence detection using biochip technology, the following analytical characteristics of the method were determined and confirmed by calculations: limit of detection, accuracy and specifity. All specified characteristics meet the standards. Therefore, the method for the detection of veterinary drugs (pharmacologically active substances) in milk and dairy products using enzyme-linked immunosorbent assay with chemiluminescent detection using biochip technology is validated.