

## Microbial contamination of cow's milk and operator hygiene

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To obtain high quality, ecologically pure milk, it is necessary to limit the possibility of access of microorganisms into it and their proliferation. From the point of view of compliance with the sanitary standards and the health of the cow's udder, the milking operator must take care of adequate hygiene throughout the technological process. In order to ensure such standards and protect animal health, a method of hand hygienic antiseptic is proposed for use, which involves the removal of mechanical contamination from the hands and treatment of their skin with a disinfectant containing ethanol - 62%, propylene glycol - 0.1%, triethanolamine - 0.2%, cross-linked copolymer of acrylic acid - 0.3%, polyethylene glycol ether alpha-tocopherol ferrol A - 0.01%, glycerol - 1.0%, diethyl phthalate - 0.08% and deionized water - 36.31%. It has been established that with a sanitary-hygienic treatment of the operator's hands before milking according to the developed method the correlation coefficient between the total point estimation of hygienic condition of hands and the level of bacterial contamination of cows' milk (CFU) decreases ( $r=+0.990$ ) ( $p<0.001$ ), which provides for the high quality product (according to the State Standard 3662:2015). To assess the hygiene quality of the milking operator hands, as a tool for determining and controlling the level of hygiene of personnel, a method has been developed that establishes a 3-point assessment, which corresponds to a universal evaluation system and allows to evaluate the quality of hygiene according to the following point scale: I - good, II - satisfactory; III - unsatisfactory.

**Keywords:** Hygiene; Purity; Milk quality; Milking operator; Disinfectant; Microorganisms

### Introduction

Milk is a valuable food and raw material for the production of a wide variety of dairy products. Therefore, it is extremely important that the milk is of good quality and ecologically pure, and does not lose its natural properties as long as possible.

The quality and ecological compliance of milk directly depends on the breed, feeding, physiological state, and conditions of keeping and technology of milking the cows (Osipenko et al., 2018; Palii et al., 2019a). Milk, being an excellent nutritional product for humans, at the same time serves as a good growth medium for the reproduction of various microorganisms, including pathogens.

Around the world, microbial contamination of milk is receiving particular attention (Msalya, 2017; Karmaker et al., 2020). This is due to the fact that the proliferating microorganisms destroy the biologically valuable substances inherent in milk.

Therefore, preventing the ingress of microorganisms into milk is a task of top priority in obtaining ecologically friendly, pure milk of high quality. The quality of milk according to its bacterial contamination largely depends on the observance of sanitary standards at all stages of its obtaining, processing, storage and transportation (Palii & Palii, 2019; Szyda et al., 2019; Velazquez-Ordóñez et al., 2019). Microbial contamination of milk occurs along all its way from producer to consumer. The rate of accumulation and dynamics of the proliferation of certain types of microorganisms depend on the sanitary status of potential sources of contamination of the milk and its storage conditions. The leading role is played by the primary microflora, which enters the milk on the farm and initially determines the quality and preservation of the product as the earliest and long-lasting contaminant. The main sources of microbiological and mechanical contamination of milk are the udder and skin of an animal, feed, flies and the equipment, as well as the personnel (Paliy, 2019; Paludetti et al., 2019; Raza & Kim, 2017).

The scientists (Fischer et al., 2015; Palii et al., 2019b) agree that milk that comes directly from the udder of a healthy animal contains a small number of microorganisms and is virtually sterile. Immediately after milking, it is gradually contaminated at all technological stages of production and processing. Milking occupies a highly important place in dairy farm management which aims

to improve the health, efficiency of cows and the quality of milk. The ability to influence the biology of cows to produce high quality milk and achieve optimal milking is partly realized through the use of milking technology and procedures (Nanka et al., 2018). As noted by Kitikov & Romaniuk (2017), the implementation of the rules of personal and industrial hygiene is of paramount importance in obtaining environmentally friendly and milk of good quality. It's of utmost importance that every employee knows the farm personal hygiene rules and observes them steadily. These rules consist of the following requirements: cleanliness of personal and sanitary clothing, care of the body, hands, hair, compliance with established sanitary regime at work, treatment of the udder of cows and dairy equipment.

From the point of view of compliance with the sanitary regulations and the health of the cow's udder, a milking operator must take care of adequate hygiene throughout the technological process. When the teat canal is opened during milking, the animal's natural udder barrier is inactive and literally open. At this moment, the milking operator is a potential carrier of germs of all kinds, as his contaminated hands are a dangerous accumulator of pathogens. During the milking process, the operator may inadvertently touch of a dirty place with his hand and immediately touch of the cow's udder with it; thus the bacteria that cause mastitis disease can move from one cow to another. At the same time, non-compliance with hygiene rules can lead to a decrease in the quality of the milk produced (Doyle et al., 2015; Swapon et al., 2017). The work of such scientists has been devoted to the study of hygiene in dairy cattle breeding and the production of high-grade, ecologically pure milk on farms and at industrial complexes (Bava et al., 2017; Miller et al., 2015; Robles et al., 2019; Sklyar et al., 2017). But despite the success of the research work on providing proper hygienic status in dairy cattle breeding and obtaining milk of adequate quality, the task of ensuring the control of hygiene quality assessment by using modern highly effective means and methods remains unsolved.

Therefore, the process of ensuring the purification of milking operator hands and control in the assessment of hygiene are one of the most important technological operations, the effectiveness of which, to some extent, depends on the sickness rate of mastitis in the actual herd and the quality of milk obtained, which gave a reason to research in this direction.

## Materials and Methods

The purpose of the research was to develop an innovative technological approach to the hygienic antiseptics of the milking operator's hands, based on the use of effective means and evaluation of the quality of his hygiene. To visually determine the quality of the operator's hand hygiene and quantify the values of microbial proliferation on the nutrient medium, the studies were performed under the same conditions, suitable for comparison and collation. Scientific and economic research was carried out during milking of high-yielding cows of the Ukrainian black-rumped dairy breed. In determining the quality of milk, we were guided by the requirements of actual normative regulations: the sampling was carried out in accordance with the State Standard ISO 707:1997; the number of microorganisms was determined according to the State Standard 7357:2013 and the State Standard IDF 100B:2003. The total bacterial contamination of the milk was investigated by a method based on the properties of mesophilic aerobic and optional anaerobic microorganisms to proliferate on a dense nutrient agar at a temperature of  $30 \pm 1^\circ\text{C}$  for 72 hours. The system of techniques to ensure the accuracy of assessment of the milk quality included the sampling of raw milk, the use of modern instrumental methods, the development of rapid methods of determining the milk quality and confirming the accuracy of their measurement. In the development of the point scale, the gradation was determined depending on the task, the accuracy required, promptness of obtaining results and the possibility of interpreting the characteristics of quality levels and purity indicators. To determine the quality of milk the device 'Ecomilk' KAM 98/2A № 271001/04 was used according to the State Standard 23453-90 and the State Standard 30518-97. It was used to analyze the quality indicators of milk composition.

The results of the studies were processed by the method of variational statistics based on the calculation of arithmetic mean ( $\bar{X}$ ), deviation from the arithmetic mean error ( $S_x$ ) and the reliability of the difference between the compared indicators ( $p$ ). Data were processed by Microsoft Excel software.

## Results and Discussion

To effectively ensure proper hygiene of milking operators, a method has been developed that involves removing mechanical impurities from the hands and treating the skin with a disinfectant containing ethanol - 62%, propylene glycol - 0.1%, triethanolamine - 0.2%, cross-linked copolymer of acrylic acid - 0.3%, polyethylene glycol ether alpha-tocopherol ferrol A - 0.01%, glycerol - 1.0%, diethyl phthalate - 0.08% and deionized water - 36.31% under an exposition of 1 min.

Table 1 contains the results of the results of the field test of the proposed method.

**Table 1.** The results of the field test of the method developed.

Chemicals proposed	Exposition	Results	
		test	Control
ethanol - 62%	5 s	+	+
propylene glycol - 0.1%	10 s	+	+
triethanolamine - 0.2%	15 s	+	+
cross-linked copolymer of acrylic acid - 0.3%	30 s	+	+
polyethylene glycol ether alpha-tocopherol ferrol A - 0.01%	1 min	-	+
glycerol - 1.0%	2 min	-	+
diethyl phthalate - 0.08%	3 min	-	+
deionized water - 36.31%	4 min	-	+
	5 min	-	+

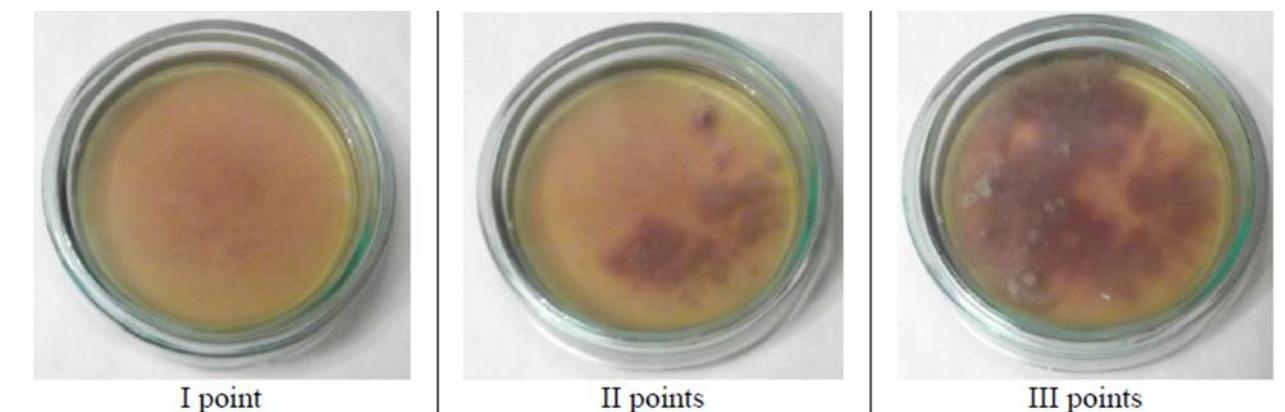
**Note:** + - Microbial proliferation is present; - - Microbial proliferation is absent.

It can be seen from the data in Tab. 1 that the disinfectant containing ethanol - 62%, propylene glycol - 0.1%, triethanolamine - 0.2%, cross-linked copolymer of acrylic acid - 0.3%, polyethylene glycol ether alpha-tocopherol ferrol A - 0.01%, glycerol - 1.0%, diethyl phthalate - 0.08% and deionized water - 36.31% under an exposition of 5-30 s doesn't show any bactericidal properties against the natural microflora of the skin of the hands.

In addition, it has been established that the specified disinfectant under an exposition from 1 min completely disinfects the natural microflora of the skin of the hands of employees.

After testing the developed method of hygienic hand antiseptic in industrial conditions, it can be stated that it meets the requirements of biosafety and biosecurity, is environmentally friendly, highly efficient and cost-effective, and also allows to reliably disinfecting the skin of the hands of the personnel.

In order to evaluate the hygiene quality of the milking operator's hands, a method has been developed which is as follows: after cleaning the hands of the operator of machine milking (washing with running water with the use of detergents) a fingerprint is made on the nutrient medium contained in a Petri dish. Then the quality of hygiene of the hands of the milking operator is evaluated according to the proliferation of microorganisms. The indicator is determined in points. Figure 1 shows the level of the milking operator's hands' contamination according to the point assessment developed.



**Figure 1.** Visual distribution of the milking operator's hands' cleanness according to the point assessment.

The interpretation of the data obtained is carried out according to Table 2.

**Table 2.** Point assessment of the milking operator hands hygiene

Scale	Microbial proliferation on a nutrient medium	Area of cell-culture dish with microbial proliferation, %
I - Good	Proliferation is absent	–
II - Satisfactory	Proliferation is insignificant	< 5
III - Unsatisfactory	Proliferation is significant	≥ 5

The method of assessing the hygiene quality of the milking operator's hands during testing has proved its effectiveness, it is easy to use, and it allows to reduce material costs for research and to prevent the transfer of bacteria from one cow to another. The developed method involves the use of cheap means; the distribution of the cleanness of operators hands by category provides reliable data. Along with this, it is extremely important to have information and to determine the relationship between the hygiene of the milking operator's hands and the level of bacterial contamination of hands and milk. In this regard, a field test was carried out to assess the quality of cow's milk, depending on the degree of contamination of the milking machine operator's hands. The studies were conducted with the involvement of 6 milking machine operators. In order to ensure a high level of sanitary and hygienic level of workers the developed method of hand hygiene was applied. The analysis of the results of studies of the degree of contamination of the lavages from the milking operators' hands by the developed score and the level of the bacterial contamination of cow's milk (CFU), allowed to establish the average numerical values of these indicators and their compliance with the milk grade according to the State Standard 3662:2015 (Table 3).

**Table 3.** The effect of milking operator's hand condition on the bacterial contamination of milk, ( $\bar{X} \pm S_{\bar{x}}$ ),  $n=6$ .

Point	Pollution of the lavage from the operator hands, mg/L	Milk CFU per cm <sup>3</sup>	Quality of milk according to the State Standard 3662:2015
I	18.0 ± 1.56	89.0 ± 9.0*	Extra fine grade
II	43.0 ± 2.41	255.0 ± 15.0	Top grade
III	78.0 ± 3.64***	356.0 ± 56.0***	First grade

\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ .

Thus, when assessing the cleanness of the operator's hands in I point, 18.0 ± 1.56 mg/l of dirt was revealed in the lavage and the milk had a bacterial contamination level of 89.0 ± 9.0 CFU thous./cm<sup>3</sup>, which corresponded to Extra Fine grade according to the State Standard 3662:2015. When assessing in II points, 43.0 ± 2.41 mg/l of dirt was revealed in the lavage, which is 25 mg more than in the one assessed in I score, and the milk had a bacterial contamination level of 255.0 ± 15.0 CFU thous./cm<sup>3</sup>, which corresponded to the Top grade. The assessment of hand cleanness in III points corresponded to the presence of 78.0 ± 3.64 mg/l of dirt in the lavage and high rate of contamination (356.0 ± 56.0 CFU thous./cm<sup>3</sup>) in the milk, which is 267 CFU thous./cm<sup>3</sup> higher than with the I point, and the milk corresponded only to the First grade according to the State Standard 3662:2015. Thus, as the estimation of the hygiene assessment of the milking operator's hands from I to III points approaches, the contamination level is

increased by 4.3 times at  $p < 0.001$ . Evaluation of the cleanness of the milking operator's hands according to the developed 3-point scale was highly probable, both by the results of the lavages obtained from the hands and by the level of bacterial contamination of the milk ( $p < 0.001$ ). Ensuring the hygienic cleanness of the milking operator's hands to the assessment of I and II points, using the developed method, confirms its effectiveness in production conditions.

It has been found that the correlation coefficient has the highest value ( $r = +0.995$ ) between the total score of the hygiene of the milking operator's hands and the level of mechanical impurities of the lavage from the hands. Considering that before milking the milking operator's hands are subjected to sanitary-hygienic treatment in the developed way, the correlation coefficient between the total score of hygiene and the level of bacterial contamination of cow's milk (CFU) slightly decreases ( $r = +0.990$ ). In addition, it has been found that the level of bacterial contamination of milk is affected by the level of contamination of the milking operator's hands ( $r = +0.972$ ). Therefore, studies using the developed methodological approach to assess the hygiene of the milking machine operator's hands have confirmed the hypothesis of a close correlation and the direct dependence of quality indicators of milk on the levels of mechanical impurities and bacterial contamination of the milking machine operator's hands, which determines the need and feasibility of using the developed method in production conditions.

Milking the cows is one of the most important processes for the treatment of animals on dairy farms. So the milking operator directly interacts with the cow's body and with its complex reflex-secretory system. One can judge on the level of technological and technical culture of the farm and milk quality from how far the employee takes into account the physiological features of the animal organism, fulfills hygienic requirements and how timely and promptly milking is carried (Paliy et al., 2018).

Milk should be obtained from healthy animals on farms that are safe as regards infectious diseases. Milk quality must meet current standards and regulations governing food quality and safety requirements (Abunna et al., 2019; Shkromada et al., 2019). The content of microorganisms in raw milk reflects the level of hygiene of its production. On farms and at industrial complexes, during milking cows special attention should be paid to the hygiene of the personnel. Otherwise, neglecting and breaking hygiene regulations can be a source of infectious disease of the udder and adversely affect the health and productivity of animals (Paliy, 2017).

Along with increased personal safety measures and the use of specialized overalls, gowns and gloves, the "mobile" nature of the work of these employees should be taken into account, as well as the likelihood of incomplete protection of protective equipment in the event of wear or malfunctioning, requiring timely and careful handling of hands. World practice shows (Muhammad et al., 2013) that good hand hygiene is performed in only 40%, i.e. out of 10 cases in which only four are needed. This is due, first of all, to the lack of sufficient knowledge and skills in hand processing techniques and the necessary motivation in the staff. For other reasons, it may be noted that there is not enough time, the lack of necessary conditions, the presence of problems with the skin of the hands, as well as insufficient financing of this area and, as a result, the acquisition of substandard antiseptics (Petrov et al., 2016).

During hand hygiene (hand washing), certain techniques must be adhered which includes the treatment all areas of the hands skin (Paliy, 2016). Often, when the plan of action is violated before milking, certain areas of the skin remain contaminated. Thus, during hand hygiene, the following areas of the skin are most often missed: fingertips (most contaminated, since all actions are performed with the tips of the fingers); areas between the fingers and thumbs (rarely exposed to hygiene). Therefore, on farms, workers must adhere to high standards of personal hygiene and properly follow its techniques. In the udder of healthy cows, milk is close to being sterile. Consequently, milk should not contain microorganisms capable of causing human disease or impairing the quality of milk and dairy products (Hadzevych et al., 2019; Modi, 2014).

The bactericidal activity of milk is due to the presence of protective agents in it, such as lactolin, lysozyme, antitoxins, bacteriolysins, immune bodies, etc. The bactericidal substances are inactivated at 90°C. During the bactericidal phase, bacterial proliferation is restrained and during this period the milk preserves its initial properties. The duration of the bactericidal phase in milk directly depends on the physiological state of the animal, the lactation period and the hygienic conditions of milk production (Ahda et al., 2018; Paliy et al., 2020; Yilma & Faye, 2013). Therefore, the most important conditions for obtaining ecologically pure milk of high sanitary quality are: cleanness at the dairy complex; compliance with conditions that prevent the entry of germs into milk in the process of milking the cows, primary processing and transportation of milk; proper treatment of milking equipment; compliance with the regulations of personal and industrial hygiene.

As noted (Paliy et al., 2020), the high level of sanitary culture of milking operators on dairy farms and complexes will ensure the stable implementation of the envisaged sanitary and hygienic regimes of hand treatment before milking and the production of high quality milk. High standards for the safety and quality of dairy products, which have become the norm for leading world producers, should be implemented on domestic farms. The prospect of further research is seen in the development of technological solutions for the cows treatment during their keeping and milking, which will ensure the health of highly productive cows, increase the output of livestock farmers and obtain the highest quality products.

## Conclusion

In order to improve the ecological situation on dairy complexes (as a tool for improving, determining and controlling the level of hygiene of the personnel), a method of hand hygiene has been developed, which involves treating their skin with a disinfectant containing ethanol - 62%, propylene glycol - 0.1%, triethanolamine - 0.2%, cross-linked copolymer of acrylic acid - 0.3%, polyethylene glycol ether alpha-tocopherol ferrol A - 0.01%, glycerol - 1.0%, diethyl phthalate - 0.08% and deionized water - 36.31%; a 3-point system was introduced to evaluate the quality of the milking operator's hands hygiene according to the following point scale: I - good, II - satisfactory; III - unsatisfactory.

It has been proved that the correlation coefficient between the total 3-point assessment of the milking operator's hands hygiene and the level of mechanical contamination of the lavage from his hands has the highest value ( $r = +0.995$ ) at  $p < 0.001$ . Considering that before milking the operator's hands are subjected to sanitary-hygienic treatment according to the developed method, the correlation coefficient between the total point assessment of the hygienic condition of the hands and the level of bacterial contamination of cow's milk (CFU) decreases ( $r = +0.91$ ) ( $p < 0.001$ ) which ensures the production of high quality products (according to the State Standard 3662: 2015).

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